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## ORIGINAL ARTICLES

### OBSERVATIONS ON RINDERPEST IMMUNISATION WITH GOAT VIRUS

BY

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(Received for publication on 20 July 1940)

#### INTRODUCTION

SINCE Edwards first effected some modification in the virulence of rinderpest virus by serial passage through goats, numerous investigators and field workers have devoted further study to the subject and such attenuated virus has now been employed in field immunisations for about the last five years. Saunders and Ayyar [1936] appear to have been among the first to have put the method to a critical and controlled test and their conclusions, working with virus which had undergone passage at Mukteswar and bovine virus adapted *de novo*, are:—

1. Judging from the mortality rate in controls there is evidence of attenuation of the virus for bovines after the 80th passage through the goat.
2. There is constancy of reaction in the experimental goats in Madras with no evidence of attenuation for these animals.
3. All the experimental goats have readily taken the infection and there is no indication so far that the virus cannot be maintained indefinitely through goats.
4. Ideal attenuation for bovines cannot be said to have been reached until the severity of reactions and the mortality rate are reduced to the level generally considered necessary for the serum simultaneous inoculation of these animals.

Among accounts of field trials of the method may be quoted Joshi [1935] who states that reactions in cattle were satisfactory and no spread to 'in contacts' was reported. Buffaloes are highly susceptible to goat virus and the serum simultaneous method was more satisfactory. Haji [1935] records that 277 oxen and buffaloes were inoculated with goat virus from local goats inoculated with Mukteswar virus. Mild temperature reactions, insufficient to prevent work, but no mortality occurred. A herd of 24 'in contact' cattle did not contract rinderpest. Kerr [1935] records a loss of only  $\frac{1}{2}$  per cent in cattle in the field. In milch cows a herd of 100 given

goat virus alone showed a slight drop in milk yield. There was no case of a pregnant cow aborting as a result of the goat virus although a number were inoculated when over seven months in calf. Buffaloes reacted more strongly than cattle, the attempt to control this by giving half the dose seems to have had an appreciable effect. The effect of work on buffaloes after inoculation was stated to enhance the reaction, but this does not appear to apply to 'plains' buffaloes, and in bullocks it seems to have no ill effect. He concludes by saying that the results far exceeded expectations; 100,000 animals have been inoculated by this method and after eliminating those animals which were obviously inoculated during the incubation period the loss worked out at half a per cent. Bachan Singh [1939] states that the reaction in the case of tissue virus (i.e. spleen) is milder than that of blood virus both in cattle and buffaloes and in old animals it is usually imperceptible. 'In such cases the duration of immunity is bound to be of short interval, which is decidedly a disadvantage of tissue virus when compared with blood virus which on account of its potent nature of reaction confers a more lasting immunity.' Among his conclusions are noted:—A fixed virus from Mukteswar possesses a low virulence for cattle of indigenous breeds. Blood virus has been found to be safe for local-bred buffaloes of this province (Central Provinces and Berar, India). The longest period of immunity tested so far was three-and-a-half years in the case of blood virus and it is anticipated that it would be potent for longer periods. If extensive inoculations are carried out in an area on a prophylactic basis, the chances of spread from vaccinated animals to non-vaccinated animals are very remote.

In contrast to these views, however, the *Annual Report of the Madras Civil Veterinary Department* for 1938-39 states 'It was also found that positive reactions to the vaccinations could set up fresh foci of infection and this was proved by the subinoculated animals developing rinderpest' and a further quotation from the same report on the same subject is 'The goat virus alone method which has been favourably reported on in other provinces was also carried out in small areas. A wide use of this method was not advocated as it has been discredited as it produces undesirable reactions and other bad effects after vaccination. At present serum simultaneous inoculation with goat virus is giving excellent results as an outbreak stopper.' Again D'Costa [1938] says that goat virus causes a 70 per cent mortality in hill bulls and can be used alone for immunisation only on cattle which have a high degree of resistance to rinderpest.

In Burma, inoculations with goat virus alone have been widely employed and the *Annual Report of the Civil Veterinary Department* for 1938 shows that 549,564 animals were inoculated during the year; indeed this method appears to have become the routine one for rinderpest control in that country. It is due to Pfaff [1939] that the production of desiccated goat spleen has been put on a practical basis and the advantages of this more stable and reliable product are manifest. This author also states that repeated attempts to transmit the disease to cattle by contact failed and field observations support these findings. With buffaloes the position is a little uncertain and the spread of disease to uninoculated buffaloes by contact with inoculated buffaloes has been reported.

In tropical Africa Daubney [1937, 1] has examined the method and records a mortality of approximately 18 per cent when the Mukteswar virus is inoculated into grade and native cattle alone. Reactions and mortality are, however, easily controlled by a small simultaneous dose of serum or a previous dose of vaccine. In his own series of passages for the attenuation of the virus he concludes that there was a gradual suppression of symptoms from the 85th passage. Subsequently [Daubney, 1937, 2] the same author describes how at the 160th passage the Kabete virus appeared to have reached a degree of attenuation for cattle similar to that of the Mukteswar strain which has been passaged in goats since 1929. Some uncertainty with regard to the reliability of the resultant immunity exists from these Kenya trials especially when serum has been combined with the method. It is further recorded from Kenya [Veterinary Department, Kenya, 1939] that a most important feature of the method is that the mild disease due to goat virus does not appear to be contagious either to cattle or to goats and there is thus less danger of the creation of foci of infection from immunisation. It is also stated that, like all methods of conferring an active immunity, goat virus must produce a reaction to produce a result.

It is apparent from a perusal of the records of the employment of goat virus for immunisation that the inherent resistance to the disease of the animals to be immunised is a prime factor with regard to the safety of the procedure and it behoves all who contemplate its employment on a large scale to make every endeavour to assess this factor. Increasing experience of rinderpest and its control impresses on one the wide variations of such resistance that occur among susceptible species, breeds and races.

#### THE VIRUS EMPLOYED

The virus was obtained from the veterinary laboratory, Insein, Burma but had originated at Mukteswar. The exact number of passages since its first adaptation to goats could not be ascertained but the number must total many hundreds (290 passages had taken place in Burma before we obtained possession). We have maintained it by subcutaneous inoculation into locally procured goats and no special attempt has been made to prolong the passage series to obtain greater attenuation. Infective spleens have been stored in the refrigerator and goats inoculated when fresh virus was required or to ensure survival and viability of the virus. Passage injections have been carried out on an average once a month. On this account few goats have been left to recover or die and we cannot accordingly make adequate comparisons of mortality with series carried out elsewhere. Temperature reactions have been fairly constant up to a peak of 105°F (morning temperature) but other characteristic symptoms have not been striking. Dullness, falling off of appetite, a marked staring coat and a tendency to stand with legs tucked in and an arched back presenting an appearance of malaise and discomfort are apparent from the time that the temperature rises and for about a week afterwards. Diarrhoea frequently follows but definite pneumonia has been encountered but rarely. Among goats that survive loss of condition often amounting to emaciation has been rather noticeable.



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## TESTS OF VIRULENCE OF THE VIRUS CATTLE

Class I. Cattle have been obtained for testing purposes from areas which are subject to frequently recurring outbreaks of rinderpest, their susceptibility is therefore unknown but if enquiries are made with regard to past history one can generally rely on young animals being susceptible.

Class II. Animals from a part of the country which, from its situation and local stock contacts, has always enjoyed long periods of freedom from the disease and which is known to have been completely free for the last five years. Bovines from this district can be relied on to be at least 90 per cent susceptible.

Class III. An area intermediate between Class I and Class II. It enjoys freedom from infection for variable periods and at the time that a test was carried out no outbreak had been reported for three years.

Ten young bovines obtained from an area of Class I were injected with 1 c.c. of a 0.5 per cent saline suspension of fresh spleen from a reacting goat. Only one animal showed anything suggestive of a definite temperature reaction and in this case the peak temperature was only 102.8°F (fourth day). Another showed slight looseness of the bowels on the ninth day and a disinclination to feed on the eleventh day. None of the other animals showed any appreciable departure from the normal. Blood was drawn from two animals, neither of which had shown any definite reaction, on the seventh day following the injection of the goat virus and each sample was injected into two susceptible buffaloes in doses of 5 c.c. Infection occurred in every case, two of the test animals died of acute rinderpest and two recovered after severe clinical attacks. The ten young bovines were retested twenty-one days later with virulent buffalo blood and appeared to be completely immune.

The experiment shows that at least four of the ten animals were susceptible to rinderpest in the sense that they were not solidly immune as a result of previous infection and recovery.

A second experiment was carried out with ten adult cattle from an area described as Class II, i.e. the most susceptible stock available in the country. Five were injected with 1 c.c. of citrated blood and five with 1 c.c. of 0.5 per cent saline spleen suspension of a reacting goat. All gave a typical temperature curve, four showed some evidence of malaise and a little diarrhoea or softening of the faeces but the departure from the normal was transient, while one animal died on the tenth day after exhibiting symptoms of only moderate severity. It was an old, thin and weak animal and death did not appear to be attributable solely to the reaction.

With animals obtained from an area of Class III, a test on eleven cattle, of ages varying from about two-and-a-half years old to very aged animals produced four definite temperature reactions with slight indisposition for, two or three days, two indications of malaise with no temperature, while five animals, all aged ones, showed no appreciable effects whatsoever and were probably immune from a previous attack.

These tests show therefore that goat virus is safe for use without serum on cattle throughout this country and that the degree of attenuation might be said to approach the ideal. Details of these tests are set out in Table I.

## TESTS ON BUFFALOES

All the buffaloes used for these tests have been those described as Class II, that is from an area which has been free from rinderpest for five years, and they can be depended upon to be at least 90 per cent susceptible. When infected with bovine or buffalo virus they develop very severe reactions and the mortality is at least 90 per cent. They are accordingly the most susceptible stock available and in their lack of resistance remind one of the Longhorn Ankole cattle of Central Africa though the syndrome developed after infection presents some differences. Temperature reactions may be erratic and often show little relation to the severity of other symptoms or to the chance of recovery and many fatal attacks have been observed which have been quite apyretic. A typical temperature curve is similar to that seen in cattle. Lachrymation often but not invariably occurs with the formation of muco pus at the inner canthus; as, however, our buffaloes when confined often develop a non-specific conjunctivitis this symptom is not very characteristic. Dullness and suspension of appetite and rumination set in about the fifth to seventh day following infection and this is followed by profuse diarrhoea; blood and mucus appear in the excreta and the condition passes to severe dysentery with considerable quantities of pure blood being passed with the watery stools. Death may occur any time after the sixth day while some fulminating cases have been observed which died four days after infection with virus. We have been unable to demonstrate coccidia in the faeces or ejecta of dysenteric buffaloes or in the faeces of normal animals before their use for tests or experiments, either by direct smears or by flotation methods, and we have no doubt that acute dysentery can occur in the absence of these parasites, solely due to a pure rinderpest reaction. On autopsy the most striking lesions are the acute gastro-enteritis and the hæmorrhagic inflammation of abomasum, caecum and colon, often with blood extravasation into the lumen of the gut fully explains the dysentery. The above short description applies to buffaloes infected with buffalo virus or with goat virus if severe reactions follow the latter. In experiments we have injected fifteen buffaloes with goat virus using fresh citrated blood or spleen suspension in saline, with the result that eight animals died, one appeared to be immune and six recovered. Of these six, four showed severe symptoms and two moderate symptoms. These results are shown in Table II.

In addition thirteen buffaloes have been injected with goat virus that has passed one passage through a buffalo or calf. The experiments were carried out in connection with infection tests and citrated blood was the vehicle of virus. Of these thirteen buffaloes, seven died and six recovered, three showing severe symptoms and three moderate symptoms. These results are shown in Table III. This series also indicates that a single passage of goat virus through a calf or buffalo does not enhance its virulence. Of the two series combined (Tables II and III) fifteen animals died out of a total of twenty-eight, which included one immune animal, and seven of thirteen recoveries suffered severe reactions. The series of tests have perforce been spread over a period of months and different samples of virus employed, but they clearly show that at least a 50 per cent mortality might

be expected in field immunisations and that the virus is far too virulent for general use for our buffaloes without serum.

Table IV shows an attempt to arrive at the minimum dose of serum necessary to protect buffaloes against goat virus. The serum used may be described as convalescent serum, that is serum drawn after recovery, from buffaloes injected simultaneously with buffalo virus and serum. No attempt at hyperimmunisation is carried out in the production of this serum. This table also illustrates the virulence of the virus for buffaloes and indicates that serum at the rate of 1 c.c. per 3 kilos to 5 kilos body-weight (80 c.c. to 120 c.c. for a buffalo weighing 350 to 400 kilos, our average test animal) was found necessary to control reactions adequately and that even with this dosage some diarrhoea may occur.

#### THE INFECTIVITY OF ANIMALS AFTER INJECTION WITH GOAT VIRUS

As recorded in the introduction, several writers have stated that little fear need be entertained that the use of live-goat virus will infect unprotected animals or set up fresh centres of infection, by contact. We have therefore endeavoured to obtain experimental evidence supporting or refuting such statements.

We have demonstrated, by the inoculation of susceptible buffaloes, that virus is present in the blood of animals between the fifth and fifteenth days after the injection of goat virus, whether the animal shows an appreciable reaction or not and also in animals partially protected by serum. The period does not represent the end points of the presence of virus, no attempt having been made to determine such dates. We have so proved the presence of virus in the blood on nine occasions in buffaloes and on two occasions in calves. The results are set out in Table V. Urine from two buffaloes injected with goat virus has been tested on two occasions and in both cases virus was absent. Virus was shown to be present in the blood of the donor of the urine on the seventh day, the non-infective urine sample being obtained on the ninth. In the other instance the urine was tested on the tenth day with, as already stated, negative results but the blood was not tested from this animal.

Nasal secretion has been tested from two calves of about one year old with the following results. The animals were from an area subject to rinderpest but were reported never to have had the disease. They were injected with a saline suspension of goat spleen. There was no appreciable reaction in either case and when retested with virulent buffalo blood after an interval of 39 days, there was again no reaction. On the seventh day following the first injection of goat virus, the nasal cavities were washed out with sterile saline and 40 c.c. of such washings were injected into susceptible buffaloes to test for the presence of virus. Buffalo 486 which received 40 c.c. nasal washings from calf XX. developed a typical rinderpest reaction, the temperature rising to 103°F on the fifth day, diarrhoea occurring on the ninth day and blood being present in the faeces on the eleventh day. The animal, however, recovered. There appeared to be no doubt that virus was present in the nasal secretion of calf XX. In the case of calf XXI, the result was not so



clear cut, the test buffalo which received 40 c.c. nasal washings from this calf showed no temperature rise until the fourteenth day when symptoms and fever developed simultaneously and the animal died on the eighteenth day following injection. Both symptoms and post mortem were indicative of rinderpest. We have experienced irregular reactions with a prolonged incubation period with other buffaloes employed for goat virus tests but have always realised that cross infection with buffalo virus subsequent to experimental inoculation cannot be ruled out with certainty when animals are maintained at a serum and vaccine institute where virus is perpetuated. Our conclusions are, however, that virus was present in the nasal secretion of calf XX and that it was probably present in the nasal secretion of calf XXI in a minimal amount. We have not deemed it necessary to test for the presence of virus in the copious nasal secretion or in faeces mixed with blood from animals which develop the severe and acute reactions so often resulting in death and we cannot but believe, that such animals are infective to other susceptible animals.

Contact experiments in rinderpest infection may be so inconclusive that a long series of negative results would be unconvincing. We have, however, carried out a small controlled test in the nature of a field trial which we will record as well as some field experiences. Three buffaloes were tied up in close proximity to cattle which had been injected with goat virus and all used the same small stream for drinking. Temperatures were recorded and no infection resulted. This observation is of course entirely inconclusive and we merely record it.

#### FIELD TRIAL

In a district which was free from rinderpest and which, according to records, had been free for three years, all bovines employed for transport, carting or packing operations (pack bullocks are extensively employed in this region) involving movement about the area were injected with goat virus. The vaccinated animals were put out of work for fourteen days but strict control of movement was not and could not be enforced. The work was carried out from village to village and in all 2,870 bovines were vaccinated. The area about which we are speaking had provided trial bullocks for testing and the indications were that the stock was about fifty per cent susceptible. Buffaloes, female bovines and calves were not inoculated at this stage and contact between these and the vaccinated animals which used common grazings and water supply was fairly close, although the custom of the country to tether animals rather than to permit more extensive ranging does curtail contact to some extent. The whole district was carefully watched for two months and there was never any indication that any in contact animal had contracted infection. Among the 2,870 animals injected, two deaths were reported and fourteen reactions were more severe than desirable.

It appears therefore, from all the evidence available, that animals infected with goat virus and which show little or very mild reactions are definitely less infective than animals infected with a more virulent virus (the experimental evidence of the absence of virus in the urine in two tests also supports such a contention). In view of the serious nature of the re-

actions in buffaloes, however, and the evidence that virus occurs in the blood and nasal secretion we feel that it would be dangerous to be unduly complacent regarding this aspect of the problem.

#### THE EFFECT OF THE SIZE OF THE DOSE OF VIRUS

As indicated in the introduction many workers attach some importance to the size of the dose and endeavour to reduce the severity of the reaction in buffaloes by giving half the cattle dose. They often maintain that this purpose is effected by so doing. Instructions for use of the Burma desiccated goat spleen virus recommend greater dilution for use in buffaloes than in cattle. The idea is not entirely new as in 1917 Schein used a similar proceeding for the double inoculation of cattle and buffaloes in Indo-China to obtain if possible some regularity of reaction and to avoid undue severity or complications. Schein used 1 c.c. of virulent blood diluted 1 in 100 with a variable dose of serum. The method, however, does not appear to have been generally accepted or utilised. Experience with experimental infection and double inoculation with bovine virus would not lead one to expect that as crude a variation of the infecting dose as 0.5 c.c. of blood or spleen suspension for buffaloes as opposed to 1 c.c. for cattle would have any regular effect on the resulting reaction. Our experiments have borne out this anticipation (Table II). Thus, of three buffaloes vaccinated with 0.25 c.c. spleen suspension two died, while of three vaccinated with 0.5 c.c. again two died and in this case the third animal appeared to have been immune before the experiment. The tables in general bear out the contention that the size of the dose affects but little the course of the reaction. If, however, dilution is carried further and a dose bordering on the minimal infective dose is arrived at then some modification of the resultant reaction appears to occur. Some authors maintain that a dose approximating the M.I.D. will result in a lengthening of the incubation period and perhaps enhance the chances of recovery. Our experience supports this view but regularity of reaction cannot be relied on by this means and death from rinderpest has occurred with as small a dose as 0.001 c.c. of goats blood (Table II). The short series of buffaloes receiving 0.1 c.c., 0.01 c.c. and 0.001 c.c. may be worth recording in greater detail. There appeared to be some lengthening of the incubation period with the animal receiving a dose of 0.1 c.c. which showed no marked symptoms until the tenth day and with the animal which received 0.01 c.c. as he also showed little until the ninth day. In the case of the animal which received 0.001 c.c., however, symptoms commenced on the sixth day and death occurred on the nineteenth; in fact the temperature chart of this animal is very closely parallel to the control animal which received 5 c.c. of the same goat's blood. Even if in the majority of cases a very small dose increases the length of the incubation period it is by no means assured that such a prolongation does make for a greater recovery rate or a happier convalescence. There is also the disadvantage that failure to infect and so immunise may be the result of such an endeavour and the length of life or activity of a virus may be prejudiced under field conditions. A convenient working dose, many times the minimal, therefore appears best for field inoculations.

## SUMMARY AND CONCLUSIONS

1. A goat virus strain of rinderpest of an indeterminate number of passages through goats (which certainly number many hundreds) has been tested on cattle from various parts of Thailand and found to be safe for these animals. In a field trial involving 2,870 heads only two deaths were recorded and a number of cases which were described as reacting markedly and possibly more than desirable was only fourteen.

2. Controlled preliminary tests on trial lots of animals are described.

3. For the buffaloes of this country the virus is too virulent and tests indicated that a mortality up to fifty per cent might be expected.

4. Infectivity experiments are described and active virus has been shown to be present in the blood of susceptible animals, at periods between the fifth and fifteenth days after the virus injection, whether the animals react or not.

5. Virus has been demonstrated in the nasal secretion of calves during a symptomless reaction. Nevertheless a field trial indicated that the risk of infection spreading from vaccinated cattle to unprotected buffaloes is small.

6. The effect of the size of the infecting dose is discussed and it is concluded that no distinct advantage is effected in making this minute.

## ACKNOWLEDGEMENTS

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TABLE I  
*Virulence tests with goat virus on cattle*

Cattle employed	Virus	Dose	Symptoms	Result	Retest virus	Period after goat virus	Result
Ten young cattle from an area of recurrent rinderpest. Class I— No. 1	Goat 3	1 c.c. of 0.5 per cent fresh spleen suspension	Fever, 102.8° F on morning of 4th and 5th day, no clinical symptoms	Recovered	Buffalo blood virus	21 days	No reaction
	Do.	Do.	No symptoms but virus demonstrated in blood on 7th day by subinoculation	Do.	Do.	Do.	Do.
	Do.	Do.	Do.	Do.	Do.	Do.	Do.
	Do.	Do.	Looseness of bowels 9th day, inappetence 11th day	Do.	Do.	Do.	Do.
	Do.	Do.	No symptoms	Do.	Do.	Do.	Do.
	Do.	Do.	Do.	Do.	Do.	Do.	Do.
	Do.	Do.	Do.	Do.	Do.	Do.	Do.
	Do.	Do.	Do.	Do.	Do.	Do.	Do.
	Do.	Do.	Do.	Do.	Do.	Do.	Do.
	Do.	Do.	Do.	Do.	Do.	Do.	Do.
Ten cattle from an area free from rinderpest for five years. Class II— 11	Goat 34	1 c.c. citrated blood	Typical temperature curve, 103.6°F on 5th day, no other symptoms	Recovered	Buffalo blood virus	18 days	No reaction
	Do.	Do.	Typical temperature curve, 105°F on 5th day, mild diarrhoea	Do.	Do.	Do.	Do.
	Do.	Do.	Typical temperature curve, 103.8°F on 4th day, no other symptoms	Do.	Do.	Do.	Do.
	Do.	Do.	Typical temperature curve, 104.2°F on 5th day, no other symptoms	Do.	Do.	Do.	Do.
	Do.	Do.	Typical temperature curve, 105.8° F on 5th day, no other symptoms	Do.	Do.	Do.	Do.



16	17	18	19	20	Died 12th day	Buffalo blood virus	18 days	No reaction
Eleven cattle from an area of intermittent malarial fever reported free for about three years. Class III—								
C1 (aged)	Goat 108	1 c.c. of 0.5 per cent fresh spleen suspension	Typical temperature curve, 104.2° F on 5th day, diarrhea symptoms	Recovered	Not	Re-tested	...	...
C2 (4 teeth)	Do.	Do.	Typical temperature curve, 104° F on 5th day, no other symptoms	Do.	Do.	Do.	Do.	Do.
C3 (4 teeth)	Do.	Do.	Typical temperature curve, 104.8° F on 5th day, mild diarrhea throughout 2nd week	Do.	Do.	Do.	Do.	Do.
C4 (2 teeth)	Do.	Do.	Typical temperature curve, 104° F on 5th day, mild diarrhea 2nd week	Do.	Do.	Do.	Do.	Do.
C5 (aged)	Do.	Do.	No symptoms	Recovered	Not	Re-tested	...	...
C6 (aged)	Do.	2.5 c.c. citrated blood diluted 1:10 with saline	Fever 5th and 6th day, (103.2° F), no other symptoms	Do.	Do.	Do.	Do.	Do.
	Do.	Do.	Fever 4th day (103.6° F), no other symptoms	Do.	Do.	Do.	Do.	Do.
	Do.	Do.	Dull on 5th day, no other symptoms	Do.	Do.	Do.	Do.	Do.
	Do.	Do.	No symptoms	Do.	Do.	Do.	Do.	Do.
	Do.	1 c.c. saline suspension desiccated spleen 0.25 gm. in 100 c.c. saline, i.e. dose 0.0025 gm. dried spleen	No symptoms	Do.	Do.	Do.	Do.	Do.
C7 (aged)	Do.	Do.	No symptoms	Do.	Do.	Do.	Do.	Do.
C8 (2 teeth)	Do.	Do.	Typical temperature curve 104.8° F 4th day, mild indisposition for 3 days	Do.	Do.	Do.	Do.	Do.
C9 (aged)	Do.	Do.	Doubtful fever 102° F 3rd and 4th day, no other symptoms	Do.	Do.	Do.	Do.	Do.
C10 (aged)	Do.	Do.	No symptoms	Do.	Do.	Do.	Do.	Do.
C11 (8 teeth)	Do.	Do.	Fever 3rd, 4th and 5th day up to 103° F, no other symptoms	Do.	Do.	Do.	Do.	Do.

TABLE II

*Virulence tests with goat virus on buffaloes. All buffaloes employed were from an area free from rinderpest for over five years (Class II)*

No.	Virus	Dose	Symptoms	Result
2002	Goat 3	0.25 c.c. of 0.5 per cent spleen suspension	Temperature reaction 103.8°F, severe symptoms, bloody diarrhoea	Died 19th day
1987	Do.	Do.	Temperature reaction 103°F, severe symptoms, bloody diarrhoea	Died 18th day
1956	Do.	Do.	Low temperature reaction 101.6°F, severe symptoms, blood in faeces	Recovered
1997	Do.	0.5 c.c. as above	No reaction, retested with virulent buffalo blood, no reaction	Immune
1954	Do.	Do.	Low temperature reaction 101.6°F, severe symptoms, bloody diarrhoea	Died 18th day
2004	Do.	Do.	Temperature reaction 103.8°F, severe symptoms, bloody diarrhoea	Died 17th day
1/73	Goat 31	0.5 c.c. of 0.5 per cent spleen suspension	Temperature reaction 105.2°F, severe symptoms, bloody diarrhoea	Recovered
1/234	Goat 34	2 c.c. citrated blood	Temperature reaction 102.2°F, severe symptoms, bloody diarrhoea	Died 17th day
1/534	Goat 50	0.1 c.c. citrated blood (10 c.c. of 1:100 dilution)	No temperature reaction, moderate symptoms, diarrhoea 5 days	Recovered
1/491	Do.	0.01 c.c. citrated blood (10 c.c. of 1:1,000 dilution)	Temperature reaction 103°F, moderate symptoms, diarrhoea 4 days	Recovered
1/585	Do.	0.001 c.c. citrated blood (10 c.c. of 1:10,000 dilution)	Doubtful temperature reaction 101.8°F severe symptoms, bloody diarrhoea	Died 19th day
1/595	Do.	5 c.c. citrated blood from same sample as above dilutions (Control to M.I.D. test)	Temperature reaction 103°F, severe symptoms, blood in faeces	Died 18th day
1/44	Goat 57	1 c.c. of 0.5 per cent spleen suspension	Temperature reaction 103°F, severe symptoms, diarrhoea for 7 days	Recovered
2/37	Goat 58	1 c.c. of 0.5 per cent spleen suspension	Low temperature reaction 102.6°F, severe symptoms, diarrhoea 7 days	Recovered
2/487	Goat 84	1 c.c. of 0.5 per cent spleen suspension	Temperature reaction 103.8°F, severe symptoms, bloody diarrhoea	Died 14th day

Of the 15 buffaloes injected with live goat virus, there were 8 deaths, 1 immune and 6 recoveries of which 4 had severe symptoms and 2 mild to moderate reactions.

TABLE III

*Buffaloes infected with goat virus though the actual virus was obtained from a calf or buffalo, i.e. goat virus after one intermediate passage in bovine or buffalo*

No.	Virus	Dose	Symptoms	Result
0/2134 0/2076	Goat 3 after passage through calf 2	5 c.c. blood	Temperature reaction 102.2°F, severe symptoms	Died 9th day Recovered convalescence protected
		5 c.c. blood	Temperature reaction 101.4°F, severe symptoms	
0/2182 0/2114	Goat 3 after passage through calf 3	5 c.c. blood	No temperature reaction, severe symptoms, bloody diarrhoea	Died 19th day Recovered
		5 c.c. blood	No temperature reaction, severe symptoms	
0/2049	Goat 3 after passage through buffalo 0/1978	5 c.c. blood	Temperature reaction 103.6°F, severe symptoms	Died 17th day
0/2031	Goat 3 after passage through buffalo 0/1998	5 c.c. blood	Temperature reaction 103.2°F, severe symptoms, diarrhoea 7 days	Died 34th day from exhaustion
0/1973	Goat 3 after passage through buffalo 0/1976	5 c.c. blood	Low temperature reaction 102°F, severe symptoms	Died 12th day
0/2075	Goat 3 after passage through buffalo 0/1971	5 c.c. blood	Low temperature reaction 102.2°F, severe symptoms, blood in faeces	Recovered
0/2037	Goat 3 after passage through buffalo 0/2047	5 c.c. blood	Temperature reaction 104.4°F, severe symptoms, bloody diarrhoea	Died 13th day
0/2088	Goat 3 after passage through buffalo 0/2047	5 c.c. blood	Temperature reaction 102.6°F, severe symptoms, bloody diarrhoea	Died 8th day
0/309	Goat 33 after passage through buffalo 1/69	5 c.c. blood	Temperature reaction 104.2°F, moderate symptoms, diarrhoea 5 days	Recovered
1/311	Goat 33 after passage through buffalo 1/71	5 c.c. blood	Temperature reaction 104.4°F, moderate symptoms, diarrhoea 8 days	Recovered
1/305	Goat 33 after passage through buffalo 1/72	6 c.c. blood	Temperature reaction 104.2°F, moderate symptoms, diarrhoea 4 days	Recovered

Of the 13 buffaloes injected with virus, there were 7 deaths and 6 recoveries.

TABLE IV

*Buffaloes injected with goat virus and serum simultaneously (This table is included partly because some of these animals were bled during the reaction and the blood injected into buffaloes set out in Table III)*

No.	Virus	Dose	Serum	Symptoms	Result	Immunity test
1998	Goat 3	0.5 c.c. spleen suspension	20 c.c.	Temperature reaction 103.4°F, moderate symptoms, diarrhoea 5 days	Recovered	Retested after 24 days with buffalo virus.
1960	Do.	Do.	Do.	Low temperature reaction 102°F, moderate symptoms, diarrhoea 3 days	Recovered	Do.
1999	Do.	Do.	Do.	No temperature reaction, moderate symptoms, diarrhoea 6 days	Recovered	Do.
1951	Do.	Do.	Do.	Indefinite temperature reaction, mild symptoms	Recovered	Do.
1965	Do.	Do.	Do.	Indefinite temperature reaction, severe symptoms, blood in faeces	Recovered	Do.
1976	Do.	Do.	40 c.c.	Temperature reaction 102.4°F, moderate to severe symptoms, diarrhoea for 3 weeks	Recovered	Do.
1971	Do.	Do.	Do.	Low temperature reaction 102°F, severe symptoms, blood in faeces	Recovered	Do.
2044	Do.	Do.	Do.	No temperature reaction, moderate symptoms	Recovered	Do.
2013	Do.	Do.	Do.	Low temperature reaction 102°F, moderate symptoms	Recovered	Do.
2047	Do.	Do.	Do.	Blocked out reaction, virus demonstrated in blood on 7th and 14th day	Recovered	Do.
1/109	Goat 31	Do.	80 c.c.	Low temperature 102.6°F, mild symptoms	Recovered	Not retested
1/72	Do.	Do.	Do.	Temperature 103.4°F, mild symptoms	Recovered	Do.
1/89	Do.	Do.	Do.	Temperature reaction 103.4°F, moderate symptoms, diarrhoea one day	Recovered	Do.
1/108	Do.	Do.	Do.	Temperature reaction 103.4°F, moderate symptoms, diarrhoea two days	Recovered	Do.
1/47	Do.	Do.	Do.	Temperature reaction 103.8°F, mild symptoms	Recovered	Do.
1/69	Do.	Do.	120 c.c.	Temperature reaction 104.4°F, moderate symptoms, diarrhoea two days	Recovered	Do.
1/116	Do.	Do.	Do.	Doubtful temperature reaction 101.8°F, no symptoms	Recovered	Do.
1/112	Do.	Do.	Do.	No temperature reaction 101.4°F, no symptoms	Recovered	Do.
1/85	Do.	Do.	Do.	Low temperature reaction 103°F, moderate symptoms, diarrhoea 2 days	Recovered	Do.
1/145	Do.	Do.	Do.	Low temperature reaction 102°F, no symptoms	Recovered	Do.
1/71	Do.	Do.	Do.	Doubtful temperature reaction 101.2°F, no symptoms	Recovered	Do.

*Infectivity experiments*

Animal under immunisation and its reactions	Period when blood or fluid was tested and dose	Animal injected with blood	Result
Calf No. 2 injected with goat virus alone, indefinite or symptomless reaction	7th day, temperature of calf 100.4°F. Dose 5 c.c. blood	Buffalo 2134	Definite rinderpest, died 10th day
Calf No. 3 injected with goat virus alone, no appreciable reaction	7th day, temperature of calf 97.0°F. Dose 5 c.c. blood	Buffalo 2076	Definite rinderpest, recovered
Buffalo 0/1976 injected with goat virus + 40 c.c. serum	6th day, temperature 101.4°F. Dose 5 c.c. blood	Buffalo 2182	Definite rinderpest, died 19th day
Buffalo 0/1998 injected with goat virus + 20 c.c. serum	6th day, temperature 100.4°F. Dose 5 c.c. blood	Buffalo 2114	Definite rinderpest, recovered
definite thermal 103.8 mild clinical, recovered	8th day, temperature 102.2°F. Dose 5 c.c. blood	Buffalo 1973	Definite rinderpest, died 12th day
Buffalo 0/1971 injected with goat virus + 40 c.c. serum	8th day, temperature 102.2°F. Dose 5 c.c. blood	Buffalo 2049	Definite rinderpest, died 17th day
definite thermal and severe clinical, recovered	10th day, temperature 97.2°F. Dose 5 c.c. blood	Buffalo 2031	Definite rinderpest, died 34th day
Buffalo 0/2047 injected with goat virus + 80 c.c. serum	14th day, temperature 99.8°F. Dose 8 c.c. blood	Buffalo 2075	Definite rinderpest, recovered
definite thermal and mild clinical, recovered	15th day, temperature 98.6°F. Dose 5 c.c. blood	Buffalo 2037	Definite rinderpest, died 13th day
Buffalo 1/72 injected with goat virus + 80 c.c. serum	15th day, temperature 98°F. Dose 5 c.c. blood	Buffalo 2088	Definite rinderpest, died 9th day
definite thermal and mild clinical, recovered	7th day, temperature 101°F. Dose 3 c.c. blood	Buffalo 1/305	Definite rinderpest, recovered
Buffalo 1/69 injected with goat virus + 120 c.c. serum	9th day, temperature 101.2°F. Dose 5 c.c. urine	Buffalo 1/311	Definite rinderpest, recovered
typical thermal, moderate clinical, recovered	10th day, temperature 100.6°F. Dose 5 c.c. urine	Buffalo 1/301	No reaction, retested with buffalo virus, died 7th day rinderpest
Buffalo 1/71 injected with goat virus + 120 c.c. serum	7th day, temperature 100.2°F. Dose 40 c.c. saline nasal washings	Buffalo 486	No reaction, retested with buffalo virus, died 8th day rinderpest
blocked out reaction, recovered	7th day, temperature 100.4°F. Dose 40 c.c. saline nasal washings	Buffalo 479	Definite rinderpest, recovered
Buffalo 1/116 injected with goat virus + 120 c.c. serum			Result uncertain, as symptoms did not occur until 13th day when diarrhoea set in at the same time as the temperature rose, death occurred on 18th and post mortem indicated rinderpest

# A STUDY OF THE FACTORS GOVERNING THE PASSAGE OF FLUIDS THROUGH THE STOMACH OF SHEEP, PART I

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## INTRODUCTION AND LITERATURE

THE importance of study of the physiology of alimentary tract of domestic animals, as a guide to the treatment of their diseases by drug given *per os*, cannot be overestimated, particularly, as it seems to have a decisive effect on the therapeutic value of various pharmaceutical preparations. In equine and canine practice, due to simplicity of the stomachs of these animals and their close structural similarity to the human stomach, many of the principles of veterinary medicine followed are the same as in human practice. In the case of ruminants, however, the structural complexity of the stomachs and an incomplete knowledge of their physiology has complicated matters a good deal. It is now known that whereas some drugs when administered to these animals by mouth are detained in the rumen or reticulum, others under favourable conditions, pass directly into the abomasum. This phenomenon evokes special interest for the veterinarian and throws a new light on the probable efficacy of the various medicinal preparations. Quin and Vander Wath [1938] observed with the help of glass beads that substances passing into the abomasum are eliminated within 48 hours after dosing, whereas in the case of their passage into the rumen it takes 7-14 days for the substance to be passed out in small quantities. Naturally, the action of drugs passing through the rumen is delayed and they may become diluted with food or undergo other changes before reaching the intended spot. Ross [1931] in conclusion to a series of experiments on sheep states that, apart from the fundamental interest from a physiological standpoint of the course taken by fluids in traversing the ruminant stomach, it is not unlikely that such a course, whether direct to the abomasum or *via* the rumen, markedly influences the action of drugs in general taken by the mouth.

The study of the course taken by drugs through the various divisions of the stomach attracted special attention in connection with the control of helminths in sheep, with a view to make the drenches more effective against the gastro-intestinal worms. In fact, the question of dosing direct into the



abomasum has been considered necessary and an effective method must therefore be evolved before an attempt is made to work out a satisfactory chemotherapy of gastro-intestinal worms. Ordinarily, all food and water when taken by bovines pass into the rumen unless, of course, as stated by Smith [1921] water is taken in excess, when the superfluous quantity may overflow from the rumen and reticulum to the abomasum. The ability of certain drugs in solution to pass directly into the abomasum is said to be due to a reflex act in which the drug is supposed to cause a stimulation of the vagus nerve in the pharynx with the subsequent closure of the oesophageal groove. As to what extent the direct passage of the drugs into the abomasum affects their efficiency has not yet been definitely worked out. Although, Green [1918] and Veglia [1918] as a result of their studies on the chemotherapy of haemonchosis in sheep claim that it makes no difference whatever, if the drug employed reaches the abomasum in concentrated form or in small quantities from rumen; yet it has been shown by Monnig [1935] that the efficacy of treatment depends not only on the drugs used but, to an equal degree, on the route taken by them. It appears from the work of Green and Veglia (*loc. cit*) which was undertaken in connection with the Government wire worm remedy\* that the results were judged by the degree of concentration of arsenic reaching the abomasum. Since this remedy also contains a large proportion of copper sulphate which does not seem to have been taken into consideration, the interpretation cannot refute Monnig's [1935] observations. Other workers, like Ross [1934] and LeRoux [1932] also consider that the efficiency of copper sulphate against haemonchosis in sheep is due to the direct passage of this drug into the abomasum. Besides the advantage of enhancing the effectiveness of anthelmintics against gastro-intestinal worms, the dosing of drugs into the abomasum has three more points of interest in its favour, i.e.

1. The action of the undiluted drugs is speedy and effective.
2. The dose of the drug can effectively be reduced minimising the possibility of producing any toxic action.
3. There is less risk of causing tympanitis if volatile compounds are to be administered.

Thus, it is apparent that a careful study of all the factors connected with the reflex closure of the oesophageal groove is likely to bring about a great deal of improvement in the veterinary therapy in general and anthelmintic medication in particular. A review of the somewhat meagre literature on the subject, as summarised below, does not give adequate information to enable one to draw definite conclusions, as in many instances the results claimed are of a conflicting nature.

Wester [1926] was perhaps the first to study the physiology of deglutition in cattle. He was able to prove that sodium salts were effective in causing the closure of the oesophageal groove in cattle and that the reflex action was more readily stimulated in thirsty animals.

Daubney [1930] concluded that drugs administered to sheep as a drench were retained in bulk in the rumen and did not reach the abomasum and small intestine for some considerable time after digestion. He also noted that

\* Government wire worm remedy issued by the Director of Veterinary Services in the Union of South Africa for the control of haemonchosis of sheep.

capsules remained in the rumen and drugs administered through the stomach tube also failed to pass through the rumen unless the tube was passed to its fullest extent.

Sprehn [1931] records tests conducted by him with small (0.2 gm. each) tablets which he found were able to pass directly into the abomasum, but Oppermann Th. and Behrens [1932] were not able to confirm this result. In connection with the passage of fluids through the ruminant stomach, Ross [1931] has shown that the passage of fluids through various divisions of the stomach of sheep is determined, rather by the period over which the animal is deprived of water, than by the nature of the fluid given. He noted that water or drugs in solution, when administered after withholding water for 40 hours, passed in bulk into the omasum and abomasum. He failed to establish that the administration of certain mineral salts *per se* markedly influenced the passage of fluids to the abomasum. Further, his observations give no reliable indication as to whether the strength of the drugs used (sodium bicarb, sodium chloride and zinc sulphate) or the volume of fluid given influenced their passage to the various organs. In a later publication [Ross, 1934] the same author again records that the period of starvation greatly helps the passage of fluids to the abomasum and that the administration of copper sulphate has a marked effect in securing the passage of the whole or a considerable part, to the abomasum.

LeRoux [1932] observed that tabloids or drugs in capsules invariably remained in the rumen, and bulky powders followed a similar course.

Monnig and Quin [1933], while studying the physiology of deglutition in merino sheep, noted that without previous stimulation of the reflex act powders do not pass into the abomasum and the fluid consistence of the ruminal content had an influence in stimulating the reflex. Later [Monnig and Quin, 1935] the same authors made further studies and were able to conclude that copper sulphate solution stimulates the reflex closure of the oesophageal groove and under favourable conditions 0.25 per cent of the solution is able to induce the reflex. The unfavourable conditions in the authors' opinion are poor condition and dryness of the ruminal contents. They further claimed that a 10 per cent solution is necessary to overcome these conditions. The authors do not consider that preliminary starvation has a favourable influence unless the sheep are starved for such a long period that the ruminal contents become fluid. The method of administration was noticed to be of no importance. Copper salts, other than the sulphate, also produced the reflex, but related metals such as zinc or silver failed to do so. Drugs in powder form and pills reached the abomasum within 15 seconds after previous stimulation of the reflex by copper sulphate solution.

Quin and Vander Wath [1938] were able to observe that small doses (2 to 3 c.c.) of a 10 per cent solution of copper sulphate, as well as silver nitrate and nicotine when dosed on the back of the tongue, all caused very profound changes in the ruminal rythm due probably to vagal reflex.

Swales [1939] records that a simple 2 per cent copper sulphate solution in 2 ounce doses is able to stimulate the reflex closure of the oesophageal groove.

In order to study the problem in greater detail, investigations were started on sheep, at the Government Cattle Farm, Hissar, in 1939. The study is being made with the help of some drenches under variable conditions and the



results are recorded after immediately slaughtering the animal. It is contemplated to make observations on a large number of sheep, but, as the required number cannot be spared in one year, trials will be carried on for a number of years. Any result of interest obtained will be published in a serial order every year. The present paper deals with the tests made during the winter season of 1939-40.

#### EXPERIMENTAL WORK

Results claimed so far by various workers indicate that fluids are more capable of stimulating the reflex and copper sulphate solution has unanimously been declared to influence the stimulation. These two findings will form the basis of our investigation and the plan of work will include the study of the effects on the stimulation mainly of :—

- (a) The nature of the drug in solution.
- (b) The strength of the drug in solution.
- (c) The volume of the drench.
- (d) Mode of administration.
- (e) Preliminary starvation.
- (f) Equilibrium of food and water in the rumen.

An attempt will also be made to study the duration of the closure of the oesophageal groove, after it has been affected by the stimulation of the reflex and to note if all substances can pass into the abomasum if given during this interval.

#### *Test No. 1*

Seven young sheep after being starved for 15-18 hours were drenched with 4 ounces each of one per cent copper sulphate solution. Carbol fuchsin was used as an indicator to trace the passage of the drug.

Number of sheep	Breed	Mode of administration	Nature of ruminal contents	Estimated percentage of drench reaching	
				Rumen and reticulum	Abomasum
47	Lohi	Front of tongue	Semi-solid	25	75
965	Bikaneri	Do.	Do.	30	70
82	Lohi	Back of tongue	Semi-liquid	15	85
984	Hissar	Do.	Semi-solid	20	80
929	Hissar	Front of tongue	Semi-liquid	25	75
872	Bikaneri	Do.	Semi-solid	25	75
995	Hissar	Back of tongue	Do.	30	70

The drench was dosed on the back of the tongue by a specially made, narrow, long-necked bottle.

*Conclusion.*—Copper sulphate in 1 per cent solution given after 15 to 18 hours' fast passed in major portion to the abomasum.

### *Test No. 2*

Six young sheep were dosed with 2 ounces each of a 2 per cent copper sulphate solution after having been kept off food and water for 15-18 hours. Carbol fuchsin was again used as an indicator.

Number of sheep	Breed	Mode of administration	Nature of ruminal contents	Estimated percentage of drench reaching	
				Rumen and reticulum	Abomasum
87R	Bikaneri	Back of tongue	Semi-solid	0	100
968	Do.	Do.	Semi-liquid	10	90
35	Lohi	Front of tongue	Semi-solid	0	100
987	Hissar	Back of tongue	Do.	0	100
649	Cross-bred	Front of tongue	Semi-liquid	5	95
8	Hissar	Back of tongue	Semi-solid	10	90

*Conclusion.*—A 2 per cent copper sulphate solution dosed after 15-18 hours' fast passes practically as a whole to the abomasum and the mode of administration on the front or back of the tongue seems to have no effect.

The above 2 tests indicate that copper sulphate in solution has the effect of stimulating the reflex act and the intensity of stimulation is greater when a stronger solution is used.

### *Test No. 3*

Four young sheep were dosed with 2 ounces each of 2 per cent copper sulphate solution without any preliminary fasting. The animals were allowed green food in the morning upto the time of dosing and one pint of water was forcibly drenched before the dose was given, gentian violet was used as an indicator.

Number of sheep	Breed	Mode of administration	Nature of ruminal contents	Estimated percentage of drench reaching	
				Rumen and reticulum	Abomasum
808	Bikaneri	Back of tongue	Semi-solid	95	5
65R	Do.	Front of tongue	Semi-liquid	95	5
654	Do.	Back of tongue	Semi-solid	80	20
42	Hissar	Front of tongue	Do.	90	10

*Conclusion.*—Copper sulphate in 2 per cent solution given without preliminary starvation was not able to stimulate the reflex and the major portion of the dose remained in the rumen. The mode of administration had no effect.

The above three tests clearly show that in spite of the inherent quality of the drug to cause the reflex act the intensity of which depends upon the strength of the solution, it fails to influence the reflex without preliminary starvation.

#### *Test No. 4*

Five young sheep were dosed with 4 ounces each of simple water after a preliminary fast of 15-18 hours. Carbol fuchsin and gentian violet were used as indicators.

Number of sheep	Breed	Mode of administration	Nature of ruminal contents	Estimated percentage of drench reaching	
				Rumen and reticulum	Omasum
584	Bikaneri	Back of tongue	Semi-solid	95	5
964	Hissar	Front of tongue	Do.	90	10
954	Bikaneri	Back of tongue	Semi-liquid	60	40
64R	Do.	Front of tongue	Do.	75	25
69	Lohi	Do.	Semi-solid	90	10

*Conclusion.*—Simple water in 4 ounce doses given after a preliminary fast of 15-18 hours was able to pass into the abomasum in small quantities. The major portion remained in the rumen. Again there was no indication of any effect of the mode of administration.

*Test No. 5*

Eleven sheep were dosed with 4 ounces each of simple water after a preliminary fast of food and water for 38-42 hours. Different indicators were used to avoid any possibility of their producing the reflex.

Number of sheep	Breed	Indicator used	Nature of ruminal contents	Estimated percentage of drench reaching	
				Rumen and reticulum	Abomasum
845	Bikaneri	Carbol fuchsine	Semi-solid	15	85
994	Cross-bred	Gentian violet	Do.	10	90
80	Lohi	Do.	Do.	5	95
93	Hissar	Neutral red	Do.	10	90
7	Do.	Methyl violet	Do.	20	80
51	Lohi	Methyl blue	Do.	..	100
62	Hissar	Carbol fuchsine	Do.	10	90
983	Do.	Gentian violet	Semi-liquid	5	95
27	Lohi	Do.	Do.	50	50
869	Bikaneri	Neutral red	Semi-solid	..	100
58R	Do.	Do.	Do.	10	..

*Conclusion.*—The whole or greater part of simple water given in 4 ounce doses after about 40 hours' fast passed directly into the abomasum.

*Test No. 6*

Four young sheep were dosed with 4 ounces each of simple water without any preliminary fast. The animals were prepared as for test No. 3.

Number of sheep	Breed	Indicator used	Nature of ruminal contents	Estimated percentage of drench reaching	
				Rumen and reticulum	Abomasum
9	Hissar	Carbol fuchsine	Semi-solid	50	50
828	Bikaneri	Do.	Semi-liquid	95	5
50R	Do.	Do.	Semi-solid	100	..
873	Hissar	Do.	Do.	100	..

*Conclusion.*—Simple water in 4 ounce doses given without any preliminary fast remained in major portion or as a whole in the rumen and reticulum.

Tests Nos. 4, 5 and 6 clearly show that water probably possesses a slight inherent property to pass in small quantities to the abomasum. This property directly increases with the increase in the period of preliminary starvation and water in small doses (4 ounces) may pass as a whole into the abomasum if given after a 40 hours' fast.

All the tests from No. 1 to 6 indicate that preliminary starvation is an important factor in the passage of fluids into the abomasum and in cases of starvation of about 40 hours' duration, the question of stimulation of reflex for the closure of the oesophageal groove does not arise. Whether this effect of preliminary fasting is due to withholding of food or water or both is yet to be decided.

*Test No. 7*

Four young sheep were dosed with 4 ounces each of simple water after having been kept away from water for 38-42 hours. Food was allowed upto the time of dosing.

Number of sheep	Breed	Indicator used	Nature of ruminal contents	Estimated percentage of drench reaching	
				Rumen and reticulum	Abomasum
83	Hissar	Gentian violet	Solid	100	..
106	Bikaneri	Carbol fuchsine	Semi-solid	90	10
594	Hissar	Do.	Solid	100	..
130	Bikaneri	Neutral red	Do.	95	5

*Conclusion.*—Simple water in 4 ounce doses failed to reach the abomasum when the sheep were kept away from water only for 38-42 hours.

*Test No. 8*

Three young sheep were dosed with 2 ounces each of a 2 per cent copper sulphate solution. No water was allowed for 38-42 hours but green fodder was fed up to the time of dosing.

Number of sheep	Breed	Indicator used	Nature of ruminal contents	Estimated percent- age of drench reaching	
				Rumen and reticulum	Abomasum
215F	Bikaneri	Carbol fuchsine	Solid	90	10
717	Do.	Do.	Liquid	40	60
958	Hissar	Do.	Solid	80	20

*Conclusion.*—The number of animals taken was too small. Sheep No. 717 accidentally could not be held off from water. However, there was some indication that a 2 per cent copper sulphate solution was unable to influence the reflex when the animals were not allowed access to water only for 38-42 hours.

Tests Nos. 7 and 8 reveal that by withholding water alone for some hours the stimulation of the reflex act is adversely affected.

*Test No. 9*

Three young sheep were dosed with 4 ounces each of simple water. No fodder was allowed for 40 hours, but one pint of water was forcibly drenched to each animal just before the test.

Number of sheep	Breed	Indicator used	Nature of ruminal contents	Estimated percent- age of drench reaching	
				Rumen and reticulum	Abomasum
844	Cross-bred	Carbol fuchsine	Liquid	5	95
804	Bikaneri	Do.	Semi-liquid with gas	80	20
82R	Do.	Do.	Liquid	50	50



*Conclusion.*—No definite conclusion could be arrived at. Number of animals being too small further trials were considered necessary.

*Test No. 10*

Four young sheep were dosed with 2 ounces each of 2 per cent copper sulphate solution after a preliminary fast of food only for a period of 38-42 hours. Water was allowed upto the last moment and one pint of water was forcibly drenched to each animal before dosing.

Number of sheep	Breed	Indicator used	Nature of ruminal contents	Estimated percentage of drench reaching	
				Rumen and reticulum	Abomasum
115	Bikaneri	Carbol fuchsine	Liquid	20	80
104	Do.	Do.	Semi-liquid	90	10
92R	Do.	Do.	Liquid	20	80
810	Do.	Do.	Do.	30	70

*Conclusion.*—No definite result could be claimed. It was intended to carry out some more trials in order to arrive at some definite conclusions, but no more animals were available for slaughter this year.

#### DISCUSSION

It is evident from the experimental data that copper sulphate solution possesses the quality of stimulating the reflex closure of the oesophageal groove and that stimulation is more marked when a stronger solution is used, but the effect of preliminary starvation on the reflex is interesting. A 2 per cent copper sulphate solution which passes practically as a whole into the abomasum on dosing after a preliminary starvation of 15-18 hours does not behave in the same manner when dosed without previous starvation and remains partly or wholly in the rumen. It leads us to conclude that preliminary starvation favours the reflex and in unstarved animals the property possessed by certain drugs to influence the stimulation of the reflex is greatly retarded. This result is contrary to the findings of Monnig and Quin [1935] who claim that preliminary starvation does not influence the stimulation of the reflex. The effect of prolonged starvation, they say, increases the fluid consistency of the ruminal contents which favours the reflex. This observation also does not agree with our findings as we noticed that prolonged starvation (Test 5) does not alter the consistency of the ruminal contents. Further, when sheep are kept away from fodder for 38-42 hours, but access to water is allowed freely,

the ruminal contents become fluid (Tests 9 and 10), but even then there is no definite indication of this condition favouring the reflex. It has been found therefore, that preliminary starvation is necessary for an effective stimulation of the reflex and it is for the same reason that from observations made by LeRoux [1932] and Ross [1934] in connection with the parasiticide action of drugs on gastro-intestinal worms, it was claimed that the operations were more successful in the case of sheep which had been starved for some hours.

Considering the ill effects caused by prolonged starvation, Monnig [1929], however, does not favour this practice. It was also noticed by us that if sheep are allowed free grazing after prolonged starvation, cases of digestive troubles, such as tympany and impaction of the rumen, are not uncommon. An overnight fast (15-18 hours), however, is usually a routine practice and this period should not in any way produce any ill effects. There is, therefore, no harm if the sheep are fasted for 15-18 hours, and this period according to LeRoux [1932] is essential when maximum effect is to be obtained for the treatment of haemonchosis in sheep. Experiments carried out by us show that 2 ounces of a 2 per cent copper sulphate solution when dosed after this period of starvation will pass in bulk to the abomasum (Test 2).

If starvation is prolonged for 38-42 hours, it is noted that the question of the stimulation of the reflex closure of the oesophageal groove is negligible as even simple water is able to pass, in part or as a whole (Test 5) into the abomasum. When the sheep are kept away from water only for this period, the reflex fails even with a 2 per cent copper sulphate solution (Tests 7 and 8). This result confirms the findings of Ross [1934] who claims that water or drugs when given in solution, after withholding water for 40 hours, pass in bulk into the omasum and abomasum, but it is necessary that food should also be withheld for the same period. It appears that, for producing a favourable condition for the reflex, a suitable equilibrium of food and water in the rumen is essential, but further investigation must be carried out in this connection. When there is deficiency of water in the rumen, most of the water or fluid drugs, even with their ability to stimulate the reflex, are retained in the rumen (Tests 7 and 8). This confirms the findings of Monnig and Quin [1935] who observe that dryness of ruminal contents is an unfavourable condition for the stimulation of the reflex. It can, therefore, be summarised that after routine dosing of sheep for gastro-intestinal worms, the animals may be allowed to graze for some time after dosing and then given water in small quantity, as most of it will then be retained in the rumen and there would be little possibility of the dilution of the drug in the abomasum. Under these circumstances there appears to be no necessity for starving the animal after dosing.

#### SUMMARY AND CONCLUSIONS

From the experimental data recorded during the year 1939-40 it has been found that :—

1. Copper sulphate in solution is able to stimulate the reflex closure of the oesophageal groove.
2. The intensity of the stimulation of the reflex with copper sulphate solution is more marked with a stronger solution when administered under identical conditions of preliminary starvation.



3. Preliminary starvation is a favourable condition for the stimulation of the reflex, and the ability of copper sulphate solution to stimulate the reflex may be greatly retarded even with a 2 per cent solution, when the animal is not starved.
4. Water, probably, possesses a slight inherent property to pass into the abomasum which increases with the period of preliminary starvation to such an extent that 4 ounces of it when given after withholding food and water for 38-42 hours will pass as a whole or in bulk into the abomasum.
5. The stimulation of the reflex is not influenced by the mode of administration of fluids.
6. There is some indication that the equilibrium of food and water in the rumen is also a controlling factor in influencing the passage of watery fluids to the various divisions of the stomach of sheep.
7. There is no necessity to fast sheep after routine dosing for gastrointestinal worms but access to water should be allowed to a limited extent, after allowing free grazing for sometime.

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# A NOTE ON *TOXOPLASMA CANIS* INFECTION IN A SPANIEL\*

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(With Plate I)

A PURE-BRED spaniel, which had spent its life in a tick-proof kennel, was subjected to the bites of ticks (*Rhipicephalus sanguineus*) which had been previously fed on a pariah dog showing acute infection with *Babesia gibsoni*. Having failed to imbibe the infection in this manner, the spaniel received intravenously 5 c.c. of blood heavily infected with *Babesia gibsoni* and was housed with pariah dogs in a separate kennel where it succumbed to *Babesia gibsoni* infection. It developed typical symptoms of the disease, though before death it showed very rare *Babesia gibsoni* in its peripheral circulation. Microscopical examination of smears from its internal organs revealed very rare *Babesia gibsoni* but fairly numerous *Toxoplasma canis*. While it is not possible to state definitely how the infection was acquired by this animal, the following possibilities suggest themselves: (a) the batch of ticks used in the feeding experiment may already have been infected; (b) these ticks in the process of feeding on the pariah dog infected with *Babesia gibsoni* may have imbibed also the *Toxoplasma* infection, although the latter alone was transmitted to the spaniel; (c) the infection may have been contracted as a result of contact with other pariah dogs; or (d) the blood used for retest may have contained *Toxoplasma* parasites, although neither the microscopical examination of the blood prior to inoculation nor the clinical symptoms gave any indication of this.

Mello [1910] first described *Toxoplasma canis* from a dog in Turin and ascribed to its presence a pathogenic condition designated by him as toxoplasmosis and characterized by the following symptoms: marked inappetence and progressive feebleness; skin and mucous membranes presenting an anæmic appearance; body emaciation; muscles very much atrophied and abundant mucoid discharge from the orbit; elevated temperature (103.4° F) accompanied by shivering; pulse feeble and sometimes imperceptible and irregular; respiration short, jerky and abdominal; cardiac murmur on thoracic

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auscultation; vomiting of mucus; bloody diarrhoea. On autopsy the dog showed the following changes: the lungs at certain places were oedematous and showed very minute nodular lesions, at which there were accumulations of the parasites; there was hyperplasia of the interlobular connective tissue; the spleen was only slightly enlarged; the liver was enlarged and showed lesions similar to those in the lungs; the kidney was congested but the pancreas was normal; the mesenteric glands were slightly hypertrophied and found to be blood-tinged on section; the yellow marrow of the long bones was transformed into red marrow; the blood in the heart and vessels not coagulated; the erythrocyte count was 1,800,000 per cubic mm.; the blood showed anæmic changes as indicated by the presence of poikilocytes, macro- and micro-cytes and a few megaloblasts; leucopenia was marked; differential counts of the leucocytes revealed polymorphonuclear 40 per cent, mononuclear 29 per cent, transitional 15 per cent, lymphocytes 16 per cent and eosinophile 0. The parasites were found in the smears of spleen, lung, liver and bone marrow. The endoglobular forms of the parasites situated within the cytoplasm of the monocytes are described as crescent-shaped and measuring  $1-2\mu$  in length and  $0.5\mu$  in breadth. The multiplication is said to take place only by longitudinal binary fission.

Yakimoff and Kohl-Yakimoff [1911] encountered *Toxoplasma canis* in smears from the organs of some dogs. The parasite is described as having two modes of multiplication, viz. binary fission and schizogony.

Carini [1911] was able to reproduce the infection by the inoculation of suspension of liver tissue from a dog that had died of toxoplasmosis. According to his observations the organs affected were the lungs, liver and spleen. The parasite which caused these lesions was identified by him as *Toxoplasma cuniculi*. A tissue suspension of the infected organs when inoculated into pigeons and rabbits produced toxoplasmosis with typical lesions and they died within 15-16 days and 7-9 days respectively.

Carini and Maciel [1913] have described natural cases of toxoplasmosis in dogs and they are of the opinion that *Toxoplasma cuniculi* and *Toxoplasma columbae* are morphologically indistinguishable from *Toxoplasma* found in dogs. It has been remarked that, as the pathogenic picture presented by *Toxoplasma cuniculi* is identical with that in *Toxoplasma canis* infection, it is doubtful whether the two parasites should be considered as separate species.

Laveran [1913] was able to produce toxoplasmosis in a dog by inoculating the peritoneal exudate of a rat showing *Toxoplasma gondii*. He noticed corneal opacity in this dog and is of opinion that *Toxoplasma gondii*, *Toxoplasma cuniculi* and *Toxoplasma canis* are not different from one another and that they should be considered as belonging to one species.

Blanc [1917] described three cases of toxoplasmosis in dogs in Tunis and in one of these he observed conjunctivitis and corneal opacity.

Boez [1921] reports spontaneous toxoplasmosis in dogs and claims to have seen schizogony of the parasite, in addition to its usual mode of multiplication by binary fission. The schizonts, according to his observations, mostly develop extra-cellularly in the lungs in the vicinity of the alveolar capillaries. The schizonts included in the cytoplasm of the macrophages can be distinguished from the others. Transmission experiments by inoculation of pulmonary nodules gave negative results.

MacHattie [1938] has reported two fatal cases of spontaneous toxoplasmosis in dogs in Baghdad. Dyspnoea and wasting appeared to be the chief symptoms. The lungs and liver, on *post-mortem* examination, showed lentil-sized necrotic areas. *Toxoplasma* was seen within the large endothelial cells. The necrotic areas exhibited much cellular degeneration and liberation of parasites. He also described the occurrence of large pear-shaped masses of parasites in the lungs and these, he believes, have a close resemblance to 'schizonts'.

Wenyon [1939] exhibited to the Royal Society of Tropical Medicine and Hygiene various preparations illustrating the method of reproduction of *Toxoplasma* and remarked that 'reproduction in these parasites is invariably by binary fission and when appearances of schizogony occur this is due to masses of parasites being so pressed together in preparation of smears that the individuality of the parasites is lost. Similar appearances occur sometimes in smears of organs infected with leishmania. As schizogony does not occur in the case of *Toxoplasma*, the ex-erythrocytic schizonts of bird malarial parasites cannot have any connection with these parasites'.

The object of this paper is to place on record the occurrence of *Toxoplasma canis* in a pure-bred spaniel, and to describe certain stages in its development which indicate that schizogony is absent in the case of this parasite and that the only method of reproduction is by binary fission, as already stated by Wenyon. The writers would like to draw the attention of field workers to this disease, and it is hoped that the brief resumé of the literature given above will be of use to them in diagnosing the condition.

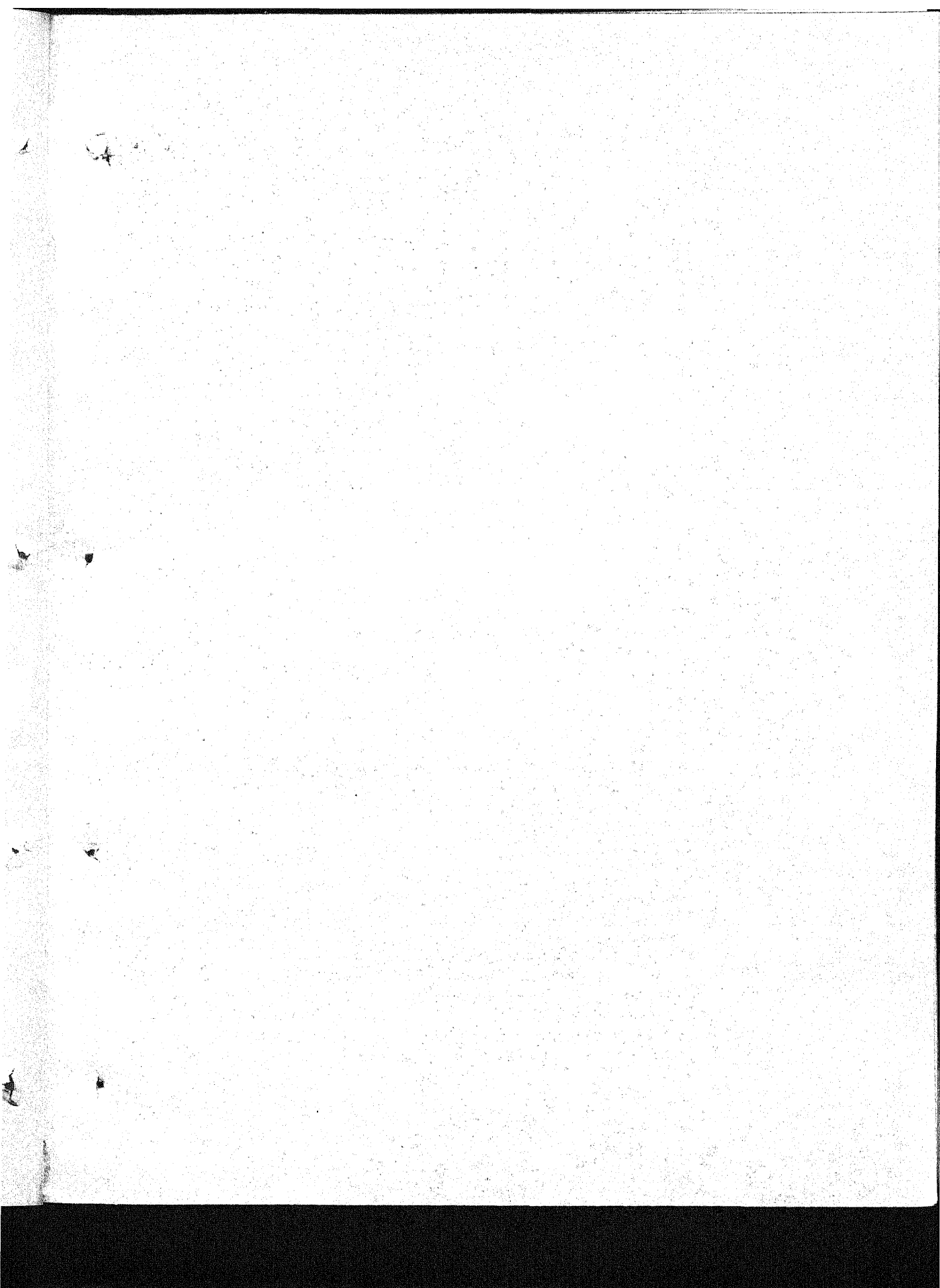
#### MATERIAL AND METHODS

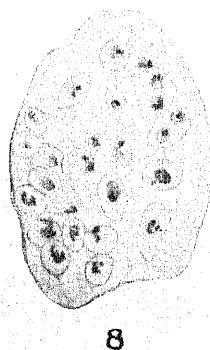
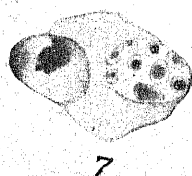
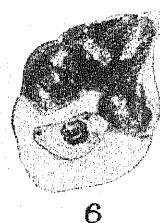
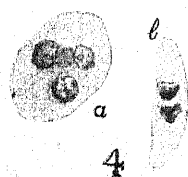
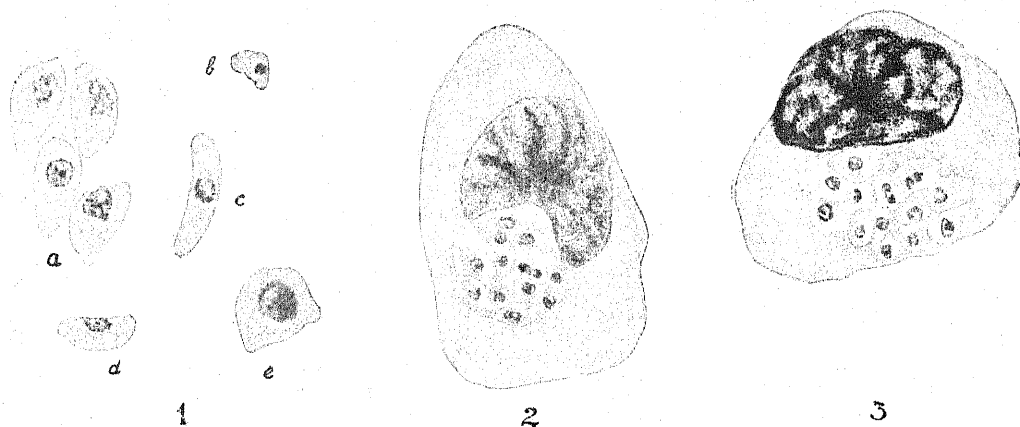
As already mentioned, the material which formed the subject of this study was obtained from the internal organs and bone marrow of a pure-bred spaniel that had succumbed to experimental infection with *Babesia gibsoni*. The organs comprised the lungs, liver, spleen and kidney. Impression preparations from the different organs were stained with May Grunwald-Giemsa. For studying the parasite in the tissues, the different organs were fixed overnight in Bouin-Duboseq and Brasil's fluid and sections were cut  $5\mu$  thick. These were stained with Heidenhain's iron-alum haematoxylin and counter-stained with chromotrop 2 R in absolute alcohol.

Careful *camera lucida* drawings were made of the various stages encountered in the preparations.

#### OBSERVATIONS

The spaniel in question had been originally subjected to bites of ticks that had been fed on dogs artificially infected with *Babesia gibsoni*. Fifteen weeks after the dog was discontinued from these experiments, it was inoculated intravenously with 5 c.c. of blood containing *B. gibsoni* parasites. From Table I it will be seen that the animal reacted as early as the third day after the infective inoculation.







## EXPLANATION OF PLATE

All figures were drawn with the aid of a *camera lucida*. Figs. 1-6 were made from air-dried smears, stained with May Grunwald-Giemsa, and figs. 7-9 were drawn from sections of spleen which were stained with Heidenhain's iron-haematoxylin and chromotrop 2 R. In fig. 8 the nucleus of the host cell is omitted. The figures are 2300 times the actual size.

- Fig. 1 .. Extracellular form of the parasite seen in smears of spleen and liver
- a .. Form showing loose nuclear structure, probably preparing for division
- b .. Youngest form with condensed nuclear structure
- c .. An elongated form with chromatinic material along the periphery of the nucleus
- d .. A young form with loose nuclear structure
- e .. A rounded parasite showing enlarged nucleus and the karyosome
- Figs. 2 and 3 .. Intracellular groups of parasite. Some of them are seen in the process of binary fission
- Fig. 4a .. Four parasites in close contact with each other
- b .. An elongated form with its nucleus already divided into two
- Fig. 5a .. An extracellular form of parasite showing two stained dots at either pole and its nucleus preparing for division
- b .. An extracellular parasite with its nucleus in metaphase
- Fig. 6 .. An intracellular parasite with two stained dots at either pole. Its nucleus is in the resting phase
- Figs. 7-9 .. Intracellular forms as seen in sections. Fig. 8 shows the nuclei of some of the parasites in the process of divisions. In figs. 7 and 9 note the central dot-like karyosome in the nucleus



TABLE I

Temperature, frequency of *B. gibsoni* and anæmic changes in the spaniel which on post-mortem examination also revealed the presence of *T. canis* in its system

Days	Temperature (°F)	Frequency of <i>B. gibsoni</i>	Anæmic changes	Remarks
1	101.6	..	..	Inoculated intravenously with 5 c.c. of virulent blood
2	101.6	..	..	Feeds well
3	102.0	rare	..	Do
4	102.2	"	..	Do
5	102.0	"	..	Do
6	101.8	"	..	Do
7	101.6	+	..	Do
8	101.8	+	..	Do
9	102.4	rare	..	Do
10	101.8	"	..	Do
11	104.4	"	..	Do
12	105.8	+++	..	Do
13	105.4	++	slight	Do
14	105.2	+++	"	Do
15	104.6	+++	++	Do
16	104.2	+++	slight	Do
17	103.8	+++	"	Do
18	104.0	++	++	Feeds fairly
19	103.8	rare	+++	Feeds fairly and dull
20	99.6	v. rare	+++	Do
21	98.0	"	+++	Off-feed, died during the night

On the twelfth day the temperature rose to 105.8°F and its blood was swarming with *B. gibsoni*. Anæmic changes in the blood also appeared on this day and these persisted until the animal died. Three days before death, however, the parasites were rarely seen in its peripheral circulation and the dog showed signs of inappetence. It died on the twenty-first night. The organ smears revealed very few *Babesia gibsoni* parasites while fairly numerous *Toxoplasma canis* were seen in various stages of development. Young extracellular forms of the parasites measuring 2-4 $\mu$  in length and 1.5-2.5 $\mu$  in breadth were seen in all the organ smears examined (Plate I, fig. 1, b); some of these extracellular forms showed signs of nuclear division and in this stage they measured 4.6 $\mu$  in length and 2.4 $\mu$  in breadth (Plate I, figs. 1, a, c, d and 4b). Intracellular forms residing in the cytoplasm of the monocytes were seen in large numbers in the smears made from the spleen (Plate I, figs. 2 and 3). Single intracellular and extracellular individuals showing signs of nuclear division were met with and, in a few instances, two pink stained dots were seen, situated at either pole of the dividing nucleus (Plate I, figs. 5a, b and 6). It is possible that these dots represented the centrosome,

because in one case a strand of fibres could be seen connecting the two dots while the dividing nucleus remained in the equatorial region. In the monocytes which contained groups of eight or sixteen parasites one could often see signs of nuclear division in some of the more well-defined individuals (Plate I, figs. 2, 3 and 8). In none of the preparations could we see a mass of cytoplasm, with nuclei embedded in it, suggestive of schizogony. Monocytes containing about thirty or more parasites were also seen and in these, too, on careful examination, one could see some of the parasites in the stage of binary fission. From these observations it is considered that schizogony is absent in *Toxoplasma* and that the only method of reproduction so far known is binary fission.

In sections of liver, evidence of marked cellular degeneration was present and embedded in this mass of degenerating cells were large numbers of extracellular parasites. A proportion of the monocytes in the sections also showed parasites and in such cases the cytoplasm of infected cells had developed a vacuole within which the parasites were crowded (Plate I, figs. 7-9). The nucleus of the parasite exhibited a vesicular appearance with a central karyosome (Plate I, figs. 7 and 9). Sections of the spleen also showed similar cellular degeneration. In the sections of the lungs and kidney, however, the parasites were not present to the same extent as in the liver and spleen.

#### SUMMARY

The occurrence of *Toxoplasma canis* in a pure-bred spaniel which had succumbed to experimental *Babesia gibsoni* infection is reported. Stages of division encountered in this parasite were suggestive of binary fission and not of schizogony. A brief resumé of the literature on toxoplasmosis in dogs is given.

#### ACKNOWLEDGEMENT

It is our pleasant duty to express our grateful thanks to Dr F. C. Minett Director, and to Capt. S. C. A. Datta, Officer-In-Charge, Veterinary Zoology Section of the Institute, for kindly going through the manuscript and for helpful suggestions.

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# THE DETERGENT EFFICIENCY OF SODA ASH, WOOD ASH AND MUD ON BRASS AND TINNED STEEL MILK UTENSILS

BY

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**T**HOROUGH cleanliness of dairy utensils is of great importance. How to clean these utensils and render them sterile soon after use at the lowest possible cost forms one of the main problems of the dairyman today. Different practices are adopted for this purpose in different countries depending on the conditions, the resources of the men in the trade and several other factors. The practice with dairies run on modern lines is that, after the preliminary scrubbing and cleaning is carried out, the utensils are sterilized either with live steam, boiling water or dry heat. The cleansing agents which are popularly known as 'washing powders' are of various kinds, namely soap powders, neutral soda, soda ash, causticized ash, scouring powders and trisodium phosphate. A solution of these powders possesses the properties of emulsifying and saponifying fat and dissolving casein. Some powders also possess abrasive properties.

In rural India the use of washing powders is practically unknown because this cannot be afforded by small milk producers. The practice throughout the country is to scour the utensils with cheap materials such as wood ash or fine mud. Scouring is mostly done on the wet utensils. After the utensils have obtained the necessary degree of polish they are washed with clean water and exposed to strong sunlight. During the rainy season, instead of exposure to sunlight, the utensils are inverted for a short time over an open fire or exposed to smoke. This practice has come down from time immemorial and is not without some technical significance. Its effect lies in the abrasive and chemical action of mud and ash and in the bactericidal action of sunlight or heat. The apparent primitiveness of the method, however, casts doubt on its efficiency and it was to determine this efficiency and to compare the method under conditions such as prevail in India with that followed by dairies adopting western practices that this investigation was undertaken.

Much experimental work has been done by foreign workers on ascertaining the efficiency of the detergent methods followed in western practice using the reagents mentioned above. The degree of the detergent efficiency of each reagent has been determined comparatively by the amount of reduction brought about in the number of bacteria. Incidentally, it may be mentioned that the term sterilization as is commonly employed in cleaning dairy equipment does not always imply absolute bacterial sterility. It means sterility to a degree good enough for practical purposes of handling milk.

Prucha, Weeter and Chambers [1918] found that 170 washed and unsteamed milk cans contained bacteria sufficient to contribute 128,000 per ml., while 91 washed and steam-sterilized cans would have added only an average



of 23,000 bacteria per ml. had the cans been filled with milk. In making a comparative study of the collective influence of 81 steam-sterilized cans with 117 unsterilized cans in which milk was handled, they found that the former added 6,800 organisms and the latter 285,600 per ml. Ayres, Cook and Clemmer [1918] found that 60 samples from sterilized pails showed an average of 6,300 organisms with a maximum of 21,500, while 59 samples from unsterilized pails gave an average of 73,300 per ml. with a maximum of 284,000. Levine, Peterson and Buchanan [1927] found that sodium hydroxide, sodium carbonate and trisodium phosphate possess germicidal properties. Mudge and Lawler [1928] have also found that alkali solutions possess germicidal property.

Determining the efficiency of cleaning utensils by the traditional method followed in India by methods used by foreign workers does not appear to have been done in this country. It was felt that this kind of work would be instructive. It was, therefore, decided to investigate the detergent efficiency of the materials used for cleaning dairy utensils in this country, namely soda ash, wood ash and mud on brass and tinned steel utensils. Since brass utensils are commonly used in rural areas and tinned utensils in the handling of milk in dairies, these two materials were selected. The investigation is not claimed to be exhaustive and complete but it is hoped that the results will illustrate the values of indigenous cleaning methods.

#### EXPERIMENTAL

(a) *Preparation of utensils.*—Four utensils of brass and four of tinned steel were used. In order to make a fair comparison, the shape, constructional features and capacity (1 litre) of the utensils were the same. All utensils were washed and steam-sterilized in a uniform manner. Five hundred ml. of well-mixed fresh milk were placed in each utensil and incubated at 37°C. to bring about souring. The utensils were emptied of coagulated milk, rinsed with 500 ml. of water, scrubbed with sterilized coconut coir and water so as to remove traces of soured milk and finally rinsed out again with 500 ml. of water.

(b) *Procedure.*—The utensils were then divided for treatment as follows :

Brass No.	Tinned steel No.	Material used for cleaning
1	1A	Control
2	2A	Wood ash
3	3A	Mud
4	4A	Soda ash

The control utensils were scrubbed with sterilized coconut coir only and rinsed with water ; the other utensils were treated similarly using the respective cleaning agents. All the utensils were then placed on a drying rack and exposed to the sun for 30 minutes.

(c) *Determining the bacterial counts.*—The average bacterial count of the water used for washing the utensils was 75 per ml. and tests for coliform organisms, *Streptococcus faecalis* and *Clostridium welchii* showed negative results in both 1 ml. and 10 ml. samples. The bacterial counts and presumptive coliform tests of all the utensils were done. For the bacterial counts,

50 ml. of sterile water was used for each control utensil and 75 ml. for each treated utensil. Standard nutrient agar was used for the plating and MacConkey's broth for the presumptive coliform tests. Plating in all cases was done with 1 ml. direct in triplicate and from this result the bacterial count of each utensil was calculated. The plates were incubated at 37°C. for 72 hours. Presumptive coliform tests were done with 1 ml. and 10 ml. in duplicate at 37°C. for 72 hours.

Each cleansing treatment under discussion was repeated 20 times in both series. The results obtained are given in Tables I and II.

TABLE I  
*Results of cleansing treatments*

Expt. No.	Brass series Number				Tinned steel series Number			
	1 (Con- trol)	2 (Wood ash)	3 (Mud)	4 (Soda ash)	1A (Con- trol)	2A (Wood ash)	3A (Mud)	4A (Soda ash)
1	6,900	3,750	6,000	2,775	16,500	7,200	5,400	3,750
2	6,750	1,650	2,250	1,500	9,900	2,850	4,500	2,700
3	7,500	2,850	3,600	1,950	12,000	2,850	2,850	1,720
4	17,250	1,875	4,875	1,725	45,000	3,450	12,750	1,500
5	16,500	1,950	4,500	2,250	60,000	1,200	2,475	975
6	15,000	1,875	5,250	1,875	75,300	3,325	4,970	2,175
7	19,500	3,150	5,525	1,350	20,000	1,650	5,700	575
8	18,450	3,450	2,700	1,275	75,000	1,125	4,875	750
9	16,800	1,200	2,250	1,650	30,450	2,250	1,875	225
10	13,650	2,775	1,725	1,050	15,750	2,170	8,250	1,220
11	14,400	1,650	2,550	1,350	62,400	1,800	3,000	1,200
12	7,050	1,275	2,175	1,200	16,500	1,200	6,700	1,350
13	13,650	3,225	5,700	2,050	67,500	2,500	7,600	1,960
14	10,800	3,525	6,600	1,350	50,800	1,800	4,250	1,550
15	16,650	1,875	3,600	1,200	49,500	3,600	4,600	2,680
16	9,750	2,475	3,000	1,875	12,750	1,950	2,525	900
17	10,500	1,950	1,800	1,125	13,500	1,425	2,925	1,050
18	7,200	2,250	2,700	975	12,300	1,295	3,750	600
19	9,000	1,800	2,175	1,200	14,250	2,170	4,200	1,200
20	11,700	2,850	5,600	1,050	16,200	1,875	3,000	1,125
Presumptive coli- form positive cases	5	1	4	Nil	8	1	5	Nil

Table I shows that on comparing with bacterial counts of the control utensils, a considerable reduction in counts in both series has been brought about and that the amount of reduction varies with cleaning material used. As regards both reduction of plate count and destruction of coliform organisms, soda ash was the most effective and next came wood ash and lastly mud.

of 23,000 bacteria per ml. had the cans been filled with milk. In making a comparative study of the collective influence of 81 steam-sterilized cans with 117 unsterilized cans in which milk was handled, they found that the former added 6,800 organisms and the latter 285,600 per ml. Ayres, Cook and Clemmer [1918] found that 60 samples from sterilized pails showed an average of 6,300 organisms with a maximum of 21,500, while 59 samples from unsterilized pails gave an average of 73,300 per ml. with a maximum of 284,000. Levine, Peterson and Buchanan [1927] found that sodium hydroxide, sodium carbonate and trisodium phosphate possess germicidal properties. Mudge and Lawler [1928] have also found that alkali solutions possess germicidal property.

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3	7,500	2,850	3,600	1,950	12,000	2,850	2,850	1,720
4	17,250	1,875	4,875	1,725	45,000	3,450	12,750	1,500
5	16,500	1,950	4,500	2,250	60,000	1,200	2,475	975
6	15,000	1,875	5,250	1,875	75,300	3,325	4,970	2,175
7	19,500	3,150	5,525	1,350	20,000	1,650	5,700	575
8	18,450	3,450	2,700	1,275	75,000	1,125	4,875	750
9	16,800	1,200	2,250	1,650	30,450	2,250	1,875	225
10	13,650	2,775	1,725	1,050	15,750	2,170	8,250	1,220
11	14,400	1,650	2,550	1,350	62,400	1,800	9,000	1,200
12	7,050	1,275	2,175	1,200	16,500	1,200	6,700	1,350
13	13,650	3,225	5,700	2,050	67,500	2,500	7,600	1,960
14	10,800	3,525	6,600	1,350	50,800	1,800	4,250	1,550
15	16,650	1,875	3,600	1,200	49,500	3,600	4,600	2,680
16	9,750	2,475	3,000	1,875	12,750	1,950	2,525	900
17	10,500	1,950	1,800	1,125	13,500	1,425	2,925	1,050
18	7,200	2,250	2,700	975	12,300	1,295	3,750	600
19	9,000	1,800	2,175	1,200	14,250	2,170	4,200	1,200
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Presumptive coli- form positive cases	5	1	4	Nil	8	1	5	Nil

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TABLE II

*Results of cleansing treatments*  
(Digest of Table I showing the results more clearly)

Particulars	Brass series Number				Tinned steel series Number			
	1 (Con- trol)	2 (Wood ash)	3 (Mud)	4 (Soda ash)	1A (Con- trol)	2A (Wood ash)	3A (Mud)	4A (Soda ash)
<i>No. of bacteria :—</i>								
Maximum	19,500	3,750	6,600	2,775	75,300	7,200	12,750	3,750
Minimum .	6,750	1,200	1,725	975	9,900	1,125	1,875	225
Average .	12,450	2,370	3,730	1,540	33,780	2,330	5,110	1,460
<i>No. of cases :—</i>								
Below 1000 bac- teria	..	0	0	1	..	0	0	6
1000 to 3000	..	15	10	19	..	17	6	13
3000 to 4000	..	5	0	0	..	2	1	1
over 4000 .	..	0	10	0	..	1	13	0
Presumptive coliform posi- tive	5	1	4	0	8	1	5	0
Order of merit	..	2	3	1	..	2	3	1

## DISCUSSION AND CONCLUSIONS

The high bacterial counts of the control utensils suggest that proper cleaning is necessary in order to keep the bacterial counts of the milk kept in them as low as possible.

There is a difference between the bacterial counts of the milk in the control utensils made of brass and tinned steel. Thus, the former contained 12,450 and the latter 33,780 organisms per ml. This is probably due to the bactericidal action of the copper or the brass on bacteria (objodynamic action).

The results stand out in favour of soda ash treatment which is recognized as suitable for cleaning purposes. Wood ash treatment did not give results as good as soda ash but closely approached it. Wood ash contains calcium oxide, hydroxide, carbonate and phosphate and also much potassium and sodium carbonate and other alkali salts. The alkali carbonates and the



scouring properties of the other constituents of wood ash contribute to this detergent action. Wood ash is thus a valuable cleaning agent and its cost is negligible. The mud treatment takes third place but still shows a considerable effect on lowering the bacterial count.

#### SUMMARY

An investigation of the cleaning powers of soda ash, wood ash and mud on brass and tinned steel milk utensils has shown that all possess detergent properties descending in efficiency in the above order.

#### ACKNOWLEDGEMENT

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# A NOTE ON *ENCEPHALITOZOON CUNICULI* INFECTION IN A RABBIT\*

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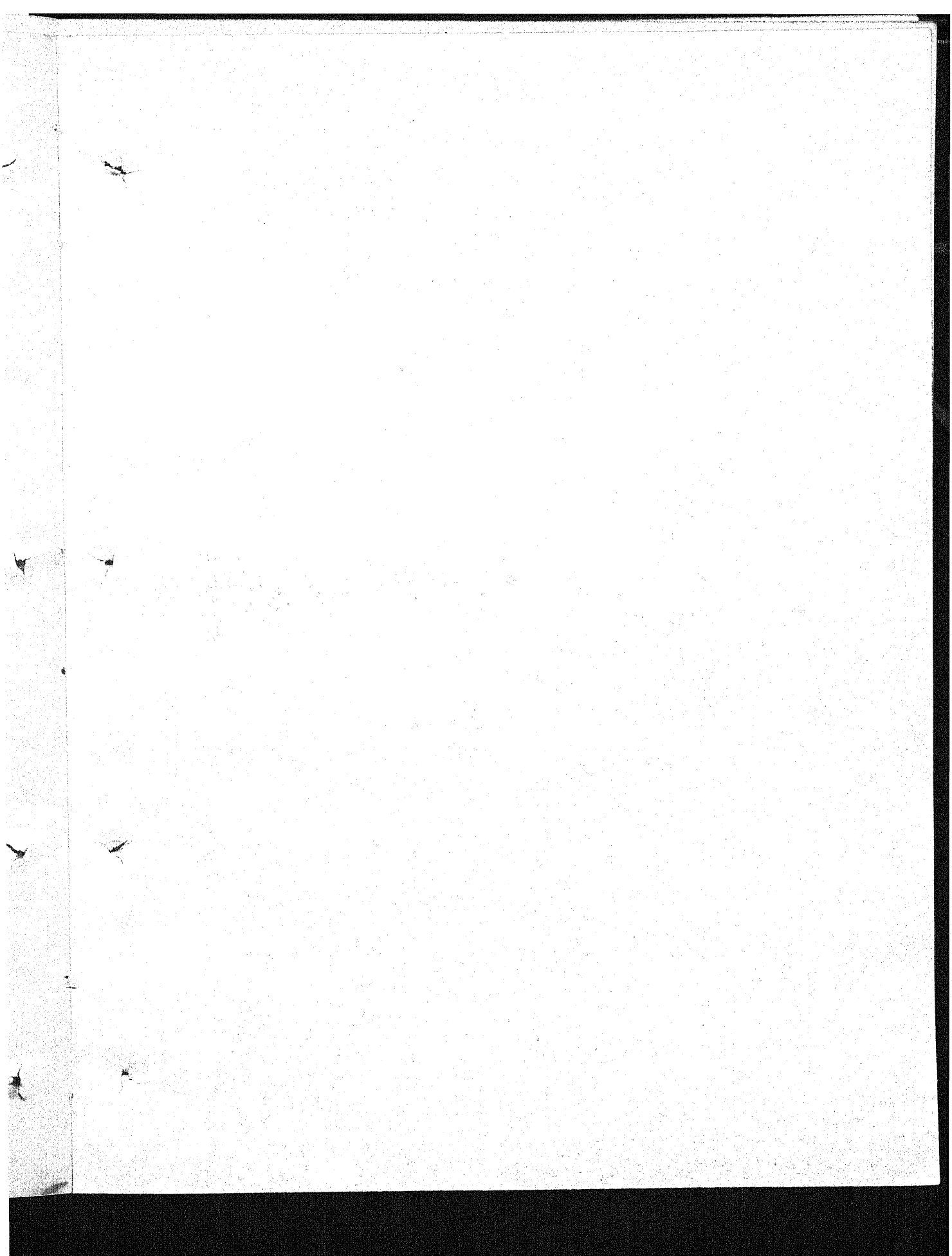
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(With Plate II)

*ENCEPHALITOZOON CUNICULI* is known to produce spontaneous encephalitis [Levaditi, Nicolau and Schoen, 1924] and nephritis [Smith and Florence, 1925] in the rabbit. Levaditi and his co-workers found the organism in the brain and kidney of the rabbit and were not only able to infect rabbits but also rats, mice and dogs. Wright and Craighead [1922] demonstrated this parasite also in the spleen, liver and myocardium. Its presence in the urine suggested to them the possible mode of infection, and later Levaditi and his collaborators were actually able to demonstrate the infectivity of the urine. Wenyon [1926], in reviewing the literature on the subject, expressed uncertainty regarding the systematic position amongst protozoa of this parasite, although Levaditi and his co-workers believed it to be a microsporidian. They demonstrated certain structures in the parasite which, they thought, were pansporoblasts, which ultimately gave rise to spores. They, however, failed to demonstrate a polar capsule and a filament in the spore. According to Wenyon [1926] their work required confirmation. It may be mentioned that microsporidia have not so far been recorded in any species of warm-blooded animal. In this article, the occurrence of *E. cuniculi* is recorded for the first time in India and a description is given of the various stages in the development of the parasite, as met with in kidney smears from an infected rabbit. Certain of these stages, when considered in their proper sequence, are suggestive of a much closer affinity of this organism with the microsporidia than with any other group of protozoa.

It should, however, be mentioned that the minute size of this organism makes elucidation of its different stages a matter of considerable difficulty. We do not claim to have seen the polar filament, but anterior to the uninucleate sporoplasm we have observed a clear area which closely resembles the polar capsule of many known microsporidia. The spore would appear to have only a single capsule, each pansporoblast giving rise to a single spore. We, however, refrain from placing it under any known family or genus of microsporidia until a more detailed study of this parasite has been carried out.

\*Paper presented to the 27th annual meeting of the Indian Science Congress, 1940.







## MATERIAL AND METHODS

The material for our observations was obtained from a rabbit which was infected with experimental trypanosomiasis. The kidneys were found to

## EXPLANATION OF PLATE

All figures, except fig. 9, were drawn from smears with the aid of a *camera lucida*. Fig. 9 was drawn from a section of kidney, which was fixed in Duboseq and Brasil's modification of Bouin's fluid and stained with Heidenhain's iron-haematoxylin and chromotrop 2 R. The smears were either air-dried or fixed in osmic vapour and stained with May Grünwald-Giemsa.

Fig. 1a Planont—found free in a smear

b, c, cl Planont—showing binary fission

d, e, f Planont—showing nuclear components for four, three and five individuals respectively

g, h, i, j Planont—showing meronts in chains ( $\times 2300$ )

Fig. 2 Urinary tubule cells showing planonts ( $\times 2300$ )

Fig. 3 A tubule cell showing schizonts ( $\times 1700$ )

Fig. 4 Tubule cell showing planonts and meronts ( $\times 1700$ )

Figs. 5, 6a and 6b Groups of meronts ( $\times 1700$ )

Figs. 7 and 8 Tubule cell showing early pansporoblasts (fig. 7,  $\times 1700$ ; fig. 8,  $\times 2800$ )

Fig. 9 Section showing the lumen of the tubule obliterated by the presence of planonts and meronts ( $\times 1166$ )

Fig. 10 Formation of spores in the cytoplasm of a tubule cell ( $\times 2300$ )

Fig. 11 Group of tubule cells showing fully-formed spores ( $\times 875$ )

Fig. 12 A portion of the same, highly enlarged ( $\times 2300$ )

Fig. 13 Tubule cell showing large number of pansporoblasts, each showing five nuclei ( $\times 1700$ )

Fig. 14 A fully-formed spore. (Note the vacuolated area at one pole) ( $\times 2300$ )

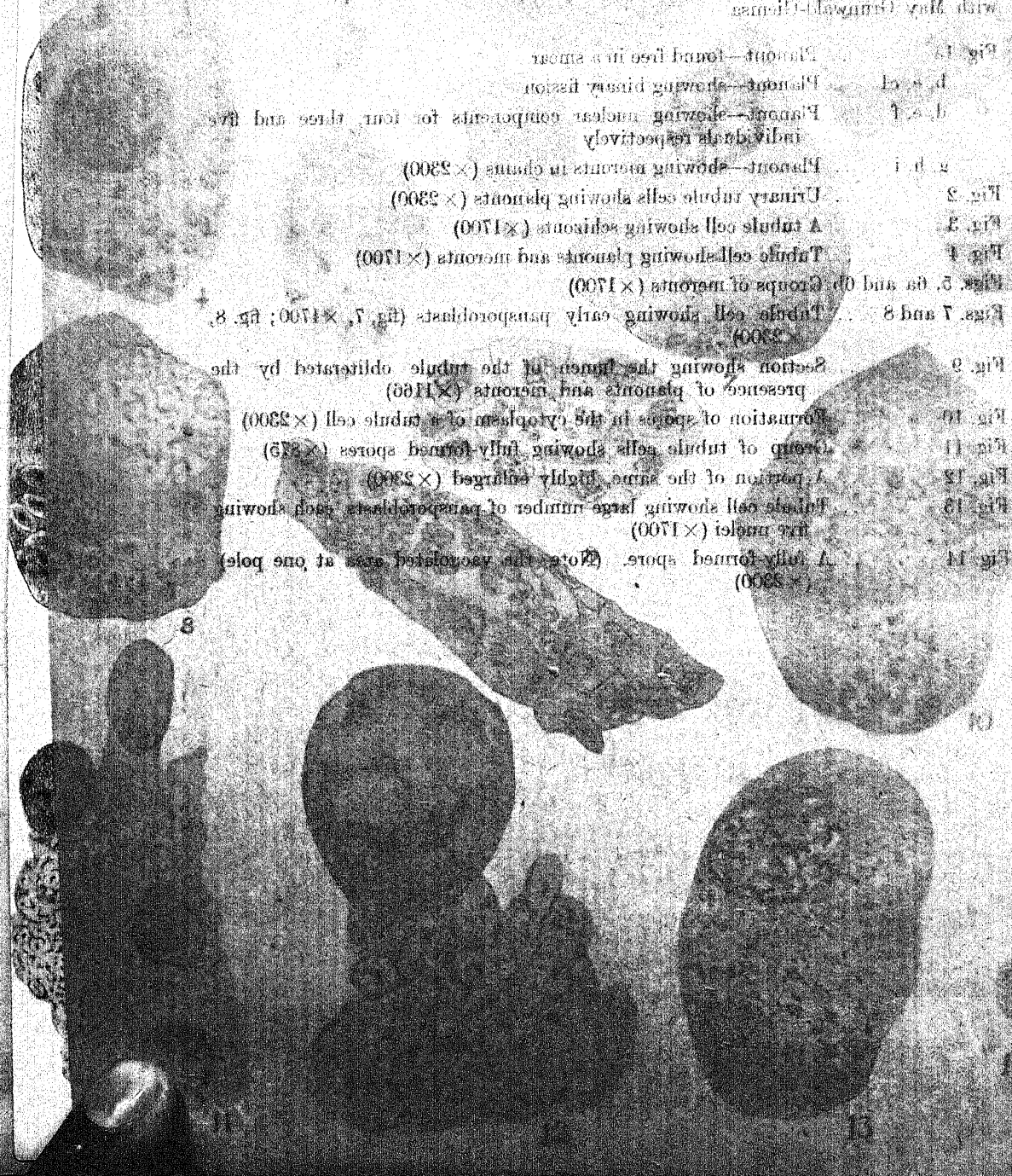
In the case of tubules, the material was fixed in Duboseq and Brasil's modification of Bouin's fluid, stained with Heidenhain's iron-haematoxylin and chromotrop 2 R. The sections were cut 5  $\mu$  thick and stained with Heidenhain's iron-haematoxylin and chromotrop 2 R. The smears were either air-dried or fixed in osmic vapour and stained with May Grünwald-Giemsa. The sections were stained with Heidenhain's iron-haematoxylin and chromotrop 2 R. The smears were either air-dried or fixed in osmic vapour and stained with May Grünwald-Giemsa. The sections were stained with Heidenhain's iron-haematoxylin and chromotrop 2 R. The smears were either air-dried or fixed in osmic vapour and stained with May Grünwald-Giemsa.



EXPLANATION OF PLATE

All figures, excepting 9, were drawn from sections with the aid of a camera lucida. Fig. 9 was drawn from a section of kidney which was fixed in Duboscq and Bial's modification of Bouin's fluid and stained with Heidenhain's iron-haematein and chromotrope 2 R. The sections were either air-dried or fixed in osmic vapour and stained with May-Grunwald-Giemsa.

- Fig. 1. Plasmot—found free in a space.  
 Fig. 2. Plasmot—showing binary fission.  
 Fig. 3. Plasmot—showing nuclear components for four, three and five individuals respectively.  
 Fig. 4. Plasmot—showing meronts in chains ( $\times 2300$ ).  
 Fig. 5. Urinary tubule cells showing plasmots ( $\times 2300$ ).  
 Fig. 6. A tubule cell showing schizonts ( $\times 1700$ ).  
 Fig. 7. Tubule cells showing plasmots and meronts ( $\times 1700$ ).  
 Figs. 8 and 9. Groups of meronts ( $\times 1700$ ).  
 Figs. 7 and 8. Tubule cell showing early pansporoblasts (fig. 7,  $\times 1700$ ; fig. 8,  $\times 2300$ ).  
 Fig. 9. Section showing the thinning of the tubule obliterated by the presence of plasmots and meronts ( $\times 1166$ ).  
 Fig. 10. Formation of spores in the cytoplasm of a tubule cell ( $\times 2300$ ).  
 Fig. 11. Group of tubule cells showing fully-formed spores ( $\times 2300$ ).  
 Fig. 12. A portion of the same, highly enlarged ( $\times 2300$ ).  
 Fig. 13. Tubule cell showing large number of pansporoblasts, each showing five nuclei ( $\times 1700$ ).  
 Fig. 14. A fully-formed spore. (Note the vacuolated area at one pole) ( $\times 2300$ ).



## MATERIAL AND METHODS

The material for our observations was obtained from a rabbit which succumbed to experimental trypanosomiasis. The kidneys were found to be slightly enlarged and on naked eye examination they showed minute petechiated areas. Impression smears made from this organ were stained in May Grunwald-Giemsa and Heidenhain's haematoxylin. Smears stained by the latter method were fixed in Schaudinn's or Bouin's (alcoholic) fluid for twenty minutes. Smears were also fixed in osmic vapour and stained with Giemsa. In order to study the organisms in sections, pieces of kidney were fixed overnight in Bouin-Duboscq and Brasil's fluid. Sections were cut  $5\mu$  thick and were stained with Ehrlich or Heidenhain's haematoxylin and counter-stained with either eosin or chromotop 2 R. Smears stained with May Grunwald-Giemsa gave a better picture of the organism than those stained with any other reagent.

Careful *camera lucida* drawings were made of the different stages encountered in the course of our study of smears and sections.

OBSERVATIONS ON THE MORPHOLOGY OF *ENCEPHALITOZOON CUNICULI*

The smallest parasites encountered in our preparations measured  $1.5\mu$  in diameter. These probably represented planonts (Plate II, figs. 1a and 2). These bodies occurred in the epithelial cells of the urinary tubules and were seen later on to become slightly amoeboid in shape, the pseudopodia being thrown out in one direction only at a time (Plate II, fig. 2). The planonts appeared to divide by two different methods, viz. (1) binary fission and (2) schizogony or multiple fission. In binary fission, two types were seen. In the first, a single meront gave rise to two daughter individuals which were more or less rounded in shape (Plate II, fig. 1, b, c, d, e, f). Groups showing sixteen to twenty meronts were often seen in our preparations. In such a group we could often see the meronts preparing for further division (Plate II, figs. 5 and 6 a, b). Some meronts also exhibited components of four individuals. The size of the meronts varied from  $2.5$ – $4.5\mu$  in diameter. In the second type the meronts assumed an elongated form and often remained in chains of four to eight individuals (Plate II, fig. 1, g, h, i). In this respect it resembled the narrow form of *Nosema apis* described by Fantham and Porter [1912]. Each individual in such a chain measured  $2\mu$  in length and  $1.1$ – $1.5\mu$  in breadth.

In the case of schizogony, amoeboid masses measuring about  $4$ – $8\mu$  in length and  $3$ – $6\mu$  across were often seen lying within the cytoplasm of the epithelial cells of the urinary tubules (Plate II, fig. 3). Sometimes the amoeboid mass of cytoplasm gave the impression of being attached to the border of the host cell. In some cases two or three such masses were seen within one cell, each mass showing a large number of nuclei. The nuclei, with a small quantity of the cytoplasm, were later seen to differentiate themselves from the amoeboid mass. Cells packed with meronts were, at times, seen to give way and thus to offer means for the parasite to escape and to infect other cells. The lumen of the urinary tubules was often obliterated by the presence of meronts or planonts (Plate II, fig. 9). Whether the

meronts resulting from schizogony underwent further schizogony or divided by binary fission was, however, very difficult to ascertain.

The next stage encountered in our preparation may be interpreted as pansporoblasts. The cytoplasm of a pansporoblast became loose in structure and the nucleus was often observed to have broken up into five very small nuclei (Plate II, figs. 7, 8 and 13). Owing to the minute size of the nuclei, it was extremely difficult to ascertain the means by which they were utilized in the process of forming a spore. In some of the preparations in which the spore wall was not fully formed, we could frequently see one or two nuclei situated at one of its poles, and it is possible that these two nuclei formed the spore wall (Plate II, fig. 10). In other preparations we could often see a nucleus situated at the margin of the vacuole which was placed anterior to the sporoplasm. Possibly this nucleus is used in the formation of the polar capsule (Plate II, fig. 10). The sporoplasm at an early stage often showed two nuclei but in the mature spore we could always find a single nucleus. The sporoplasm was seen to occupy one end of the spore, which at the other end exhibited a vacuolated area very much resembling the polar capsule (Plate II, figs. 11, 12 and 14). It may, however, be mentioned that no structure resembling a polar filament was encountered inside this so-called polar capsule. It was also observed that a single pansporoblast gave rise to a single spore. The host cell was often seen to be packed with numerous spores and in such cases cytoplasmic hypertrophy was noted, the nucleus remaining unaltered. A fully formed spore is ovoid in shape and measures  $2.2.5\mu$  in length and  $1.1.5\mu$  in breadth.

#### SYSTEMATIC POSITION

Regarding the systematic position of this parasite, it is not possible to express a definite opinion at this stage of our observations but certain characteristics of the parasite, as enumerated below, would appear to show that, as already mentioned, it has a closer relationship with the microsporidia than with any other member of the protozoa :—

- (1) the organism has an early amoeboid stage ;
- (2) multiplication takes place either by binary fission or schizogony ;
- (3) the resisting phase or spore is formed from a pansporoblast, which initially is a single nucleated individual but in the process of formation of the spore breaks up into several nuclei ;
- (4) at one end of the spore there is a vacuolated area which has a very close resemblance with the polar capsule of other microsporidia.

As, however, no polar filament was encountered inside this polar capsule it is not possible to assign this parasite to any particular group of microsporidia.

#### TRANSMISSION EXPERIMENT

Two rabbits were inoculated, one subcutaneously and one intraperitoneally, with a suspension of infected kidney tissue. These animals were kept under observation for over two months, but they failed to become infected.



## SUMMARY

*Encephalitozoon cuniculi* has been recorded for the first time in India from the kidney of a rabbit which had succumbed to experimental trypanosomiasis. Its morphology has been described in detail. The different stages of development encountered in this parasite strongly suggest its microsporidian affinity, although the polar filament was not observed. Transmission experiments carried out on rabbits were ineffectual.

## ACKNOWLEDGEMENT

It is our pleasant duty to express our grateful thanks to Dr F. C. Minett, Director, and Capt. S. C. A. Datta, Officer-in-charge, Veterinary Zoology Section of the Institute, for kindly going through the manuscript and for helpful suggestions.

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# NEW FELLODISTOMIDS (TREMATODA) FROM INDIAN FISHES

PART III. A NEW PARASITE OF THE GENUS *HAPLOCLADUS*  
ODHNER, 1911, FROM AN INDIAN MARINE FISH

BY

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(Received for publication on 7 August 1940)

(With one text-figure)

AMONGST the digenetic trematodes, the genus *Haplocladus* is remarkable in having a single intestinal caecum which opens to the outside at the posterior end. The genus was created by Odhner [1911] for two new species of trematodes *H. typicus* and *H. minor*, which he found parasitic in the gut of *Pleuronectes limanda*. In this paper, the author describes the third species of the genus which is parasitic in the intestine of an Indian marine fish.

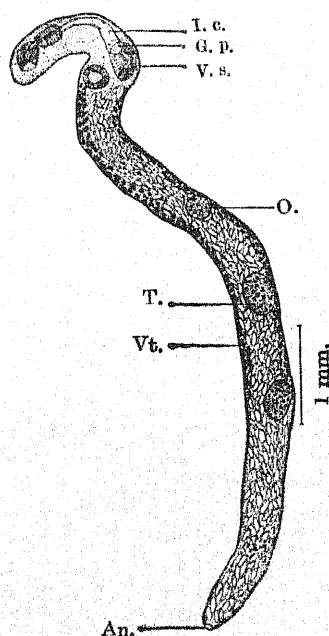
## *HAPLOCLADUS ORIENTALIS*, N. SP.

In the course of an extensive parasitological examination of the marine food fishes of the Bay of Bengal, the author recovered two mature specimens of this parasite from the intestine of *Synaptura orientalis* (Bloch and Schn). In the living state, the worms are light brown in colour and exhibit marked power of contraction and expansion. They have a narrow, elongated body which measures 6.14-7.16 mm. in length. The body is of nearly uniform breadth, 0.52-0.64 mm. and bears minute, backwardly directed, cuticular spines. The oral sucker is an inverted pear-shaped structure of 0.28-0.34 mm.  $\times$  0.22-0.24 mm. in size. It is situated sub-terminally at the anterior end of the body. It is followed by an extremely small pre-pharynx which is visible only in extended living specimens. The pharynx is an elongated oval and muscular structure, measuring 0.28-0.3 mm.  $\times$  0.12-0.16 mm. in size, and is followed by an oesophagus 0.28-0.4 mm. long. The oesophagus does not bifurcate into two intestinal caeca, but continues into a single straight, intestinal tube, which opens to the outside at the hinder end of the body. The acetabulum consists of a small, circular and poorly muscular cup-shaped structure, which measures 0.22-0.26 mm. in diameter. It is situated at the end of the first eighth or fifth of the body-length.

The testes are two in number. They are small, oval bodies, situated one behind the other. The two testes are separated by a distance of 0.42-0.7 mm. The anterior testis measures 0.34-0.38 mm.  $\times$  0.3-0.38 mm. in size and lies close behind the anterior half of the body. The posterior testis is nearly of the same size as the anterior and measures 0.3-0.4 mm.  $\times$  0.24-0.34 mm. The cirrus sac is a small, ovoid structure of



0.36-0.46 mm.  $\times$  0.28-0.34 mm. It is situated on one side of the acetabulum, with its greater part lying in front of the latter. The vesicula seminalis is bi-partite, being divided into two parts by a transverse constriction, and measures 0.17-0.18 mm.  $\times$  0.07-0.08 mm. It is continued anteriorly into a small pars prostatica, which is surrounded by prostate glands. The pars prostatica opens to the outside through a small ductus ejaculatorius. The vesicula seminalis, the pars prostatica, the prostate glands and the ductus ejaculatorius are all enclosed inside the cirrus sac.



Ventral view of *Haplocladus orientalis*, n. sp.

An. = Anus; G. p. = Genital pore; I. c. = Intestinal caecum; O. = Ovary; T. = Testis; Vt. = Vitellaria; V. s. = Vesicula seminalis.

The ovary is a small, oval or spherical body, which measures 0.24-0.26 mm.  $\times$  0.2-0.22 mm. It is pre-testicular, being situated 0.6-0.76 mm. in front of the anterior testis. The shell gland complex is diffuse and pre-ovarian. A receptaculum seminis is absent. The vitellaria consist of small, oval follicles, which extend along the side of the body from behind the acetabulum to the level of the posterior testis. The uterus is well developed and occupies the whole of the post acetabular space of the body. It contains numerous, yellowish brown operculate eggs measuring 0.034-0.038 mm.  $\times$  0.019-0.023 mm.

In the general feature of its anatomy, the new species, *Haplocladus orientalis*, described in this paper, stands nearest to the type species,

*H. typicus* Odhner, 1911. It, however, differs from the latter in the much cephalad position of the acetabulum and the extent of the vitellaria, which reach the level of the posterior testis in the Indian species, while, in the type species, they stop in front of the anterior testis. It also differs in the relative positions of the gonads. There are also differences in the measurements of the various organs.

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# NEW HEMIURIDS (TREMATODA) FROM INDIAN MARINE FOOD FISHES

## PART II. TWO NEW PARASITES OF THE GENUS *STERRHURUS* LOOSS, 1907

BY

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(With two text figures)

LOOSS [1907] created the genus *Sterrhurus*, with *S. musculus* as its type species, and included in this genus three more species: *S. imocavus*, *S. grandiporus* and *S. fusiformis*. Subsequently, twelve more species have been added to the genus: *S. monticellii*, *S. brevicirrus*, *S. inimici*, *S. floridensis*, *S. laevis*, *S. praeclarus*, *S. robustus*, *S. profundulus*, *S. branchialis*, *S. magnatestis*, *S. musigarei* and *S. magnus*. The occurrence of the members of the sub-family Sterrhurinae Looss, 1907, in Indian hosts was first reported by the author [Srivastava, 1935]. In the present paper two new species of the genus *Sterrhurus*—*S. monolecithus* and *S. karachii*—are described. The former is a very common parasite of the stomach of the Indian migratory fish, *Clupea illisha*, during winter months, while the latter is a very rare inhabitant of the stomach of a marine fish in the Arabian Sea.

Genus—*Sterrhurus* Looss, 1907.

*Sterrhurus monolecithus*, n. sp.

Host—*Clupea illisha*

Habitat—Stomach

Locality—Allahabad, Puri and Karachi

This species represents probably the most common trematode infesting Indian fishes. Large numbers of specimens of this parasite have been collected from the stomach of *Clupea illisha* examined at Allahabad, Puri and Karachi. The host is a migratory fish and is found in large numbers in the Ganges and Jumna at Allahabad, in the Bay of Bengal and in the Arabian Sea. During the winter months about ninety per cent of the hosts were found harbouring this parasite. The infestation was, however, never found to be very heavy; the maximum number of specimens obtained from a single host at Allahabad being nineteen.

The worms are light brown in colour and possess considerable power of contraction and expansion. The body is nearly cylindrical and is feebly muscular. It has a very rudimentary tail, which is visible only in fully extended specimens. Deep transverse cuticular annulations are present all over the body. Though this species varies considerably in size, the measurements given in this paper hold good for the majority of specimens. The type specimen in permanent mount measures 2.4 mm. in length, which includes a small tail of  $0.1 \times 0.16$  mm. The elongated body has a nearly uniform breadth, though it is broadest across the level of the acetabulum, where it measures

0.38 mm. The oral sucker is transversely oval in outline and measures  $0.09 \times 0.12$  mm. It is situated just behind a small preoral lip at the extreme anterior end of the body. The opening of the oral sucker is more or less anteriorly directed. The prepharynx and œsophagus are extremely rudimentary. A small, spherical pharynx 0.05 mm. in diameter is present. The œsophagus bifurcates into two, long, narrow, somewhat sinuous cæca which end blindly near the hinder end of the body proper. They never extend into the tail region. The acetabulum is a well-developed, spherical structure of 0.26 mm. in diameter. It is situated just in front of the second quarter of the body-length.

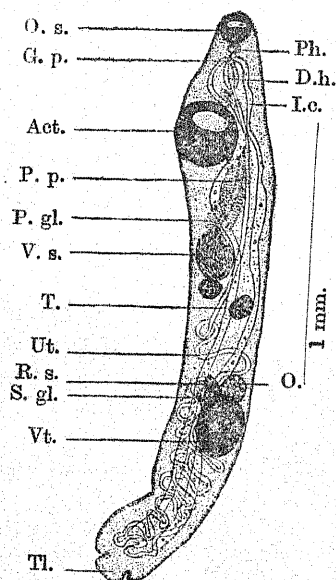


Fig. 1. Ventral view of *Sterrhurus monolecithus*, n. sp.

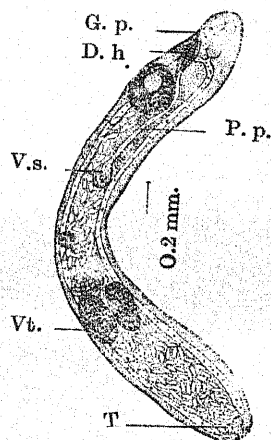


Fig. 2. Ventral view of *S. karachi*, n. sp.

Act.=Acetabulum; D. h.=Ductus hermaphroditicus; G. p.=Genital pore; I. c.=Intestinal cæcum; O.=Ovary; O. s.=Oral sucker; Ph.=Pharynx; P. gl.=Prostate glands; P. p.=Pars prostatica; R. s.=Receptaculum seminis; S. gl.=Shell gland; T.=Testis; Tl.=Tail; Ut.=Uterus; Vt.=Vitellarium; V. s.=Vesicula seminalis

The testes are two, small, oval or spherical bodies, nearly equal in size, measuring  $0.08-0.1$  mm.  $\times$   $0.06-0.08$  mm. They are situated somewhat asymmetrically, on either side of the median line, at about the equator of the body. The right testis lies slightly in front of the left. The vesicula seminalis is a fairly large, pear-shaped, thin-walled sac. It measures  $0.2 \times 0.16$  mm. and is situated just in front of the testis. It opens anteriorly into a long, tubular, more or less straight and intercæcal pars prostatica, which is surrounded by numerous prostate glands. Terminally the pars prostatica joins the distal part of the uterus, a little in front of the acetabulum, to form

a small ductus hermaphroditicus. The latter is enclosed into a small spindle-shaped, muscular hermaphroditic sac measuring  $0.1 \times 0.04$  mm. The genital pore is situated on the ventral body surface at the level of the intestinal bifurcation.

The ovary is a small, oval structure measuring  $0.1 \times 0.08$  mm. and is situated in front of and slightly overlapping the vitelline mass. The latter is a single, large, ovoid mass  $0.24 \times 0.18$  mm. in size, lying at the base of the third quarter of body-length. A small bulb-shaped receptaculum seminis is present in some specimens at the angle between the ovary and the vitelline mass. The shell gland complex lies between the median line and the anterior quarter of the vitelline mass. In fully mature specimens, the uterus occupies the whole of the post testicular area and contains a large number of yellowish-brown, operculate eggs measuring  $0.019-0.023$  mm.  $\times 0.08-0.01$  mm. In front of the testes the uterus follows more or less straight course. It never enters the tail region.

The excretory bladder is Y-shaped, with its main stem bifurcating into two lateral cornua just behind the acetabulum. The cornua unite dorsally to the oral sucker.

Amongst the species of the genus *Sterrhurus*, the new species, *S. monocithus*, is unique in having an extremely rudimentary tail and a single compact vitelline mass.

*Sterrhurus karachii*, n. sp.

Host—*Clupea longiceps*

Habitat—Stomach

Locality—Karachi, Arabian Sea

Only two specimens of this parasite were recovered from the stomach of one out of over twenty-four fish examined at Karachi, in June, 1936. The parasite has an elongated, narrow, cylindrical body which measures 1.4 mm. in length and 0.2 mm. in maximum breadth. It is of nearly uniform breadth throughout and has prominent transverse annulations all over. An extremely rudimentary tail is present. The subterminal and spherical oral sucker measures 0.08 mm. in diameter and is situated behind a prominent oral lip. A prepharynx is absent. A small pharynx  $0.05 \times 0.04$  mm. in size and an oesophagus are present. The intestinal cæca consist of two, long, narrow tubes which run along the sides of the body and end blindly at the hinder end. They never extend into the tail. The acetabulum is a well-developed, spherical structure, 0.15 mm. in diameter, and is situated at the end of the first quarter of the body-length.

The testes are two, small, spherical bodies of equal size. They measure 0.06 mm. in diameter and are situated symmetrically just behind the first half of the body. The vesicula seminalis is a pear-shaped, thin-walled sac, slightly bigger than the testes, situated in the median line a short distance in front of the latter. It is continued anteriorly into a long, narrow pars prostatica which is surrounded by a large number of prostate gland cells. The pars prostatica and the terminal part of the uterus join together to form a spindle-shaped ductus hermaphroditicus which is enclosed in a hermaphroditic sac. The genital pore is situated on a level with the anterior margin of the pharynx.



The ovary measures  $0.06 \times 0.08$  mm. and is situated just in front of the vitellaria. The latter are composed of a pair of elongated, oval, compact bodies measuring  $0.15 \times 0.08$  mm. and are situated symmetrically at the junction of the last two-thirds of the body-length. The uterus is well developed and occupies the whole of the intercæcal space behind the acetabulum. It contains a large number of operculate eggs  $0.015-0.019$  mm.  $\times$   $0.08$  mm. in size. The excretory bladder is similar to that described in the previous species.

*Sterrhurus karachii*, n. sp. resembles *S. monolecithus* in the position of the acetabulum, the almost symmetrical position and size of the testes, the long pars prostatica and the extremely small tail. But it differs from the latter species in the relative positions of the genital pore and the vesicula seminalis, in possessing two, compact, elongated, oval, symmetrically placed vitelline masses, and in marked differences in measurement. In the character of its vitellaria, *S. karachii* resembles *S. profundulus* [Manter, 1934], but differs from it in all other important features.

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# NEW HEMIURIDS (THEMATODA) FROM INDIAN MARINE FOOD FISHES\*

## PART VIII. THE MORPHOLOGY AND SYSTEMATIC POSITION OF A NEW PARASITE—*INDODERO GENES PURII*, GEN. ET. S. P. NOV. (SUB-FAMILY DEROGENETINAE)

BY

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(Received for publication on 14 June 1940)

(With one text-figure)

IN 1933 the author described two new species and a new variety of the genus *Halipegus* Looss, 1899, two new species of the genus *Progonus* Looss, 1899, and two species of a new genus *Ophiocorchis*, parasitic in Indian hosts. The synonymy between the genera *Vitellotrema* Guberlet, 1928, and *Halipegus* was fully discussed and the suppression of the former name was suggested. The systematic position of the sub-family Derogetinae Odhner, 1927, was discussed and all the above parasites were assigned to it. In a subsequent paper [Srivastava, 1934], an errata to the above paper was published in which it was pointed out that one of the points of difference between the genera *Halipegus* and *Derogetes* was the presence of a polar filament in the eggs of the former genus. The identity between the genera *Genarchella* Travassos, Artigas and Pereira, 1928, and *Halipegus* was also pointed out and it was suggested that the former genus should be considered synonymous with the latter.

Manter [1938] includes the following genera under the sub-family Derogetinae: *Bunocotyle* Odhner, *Derogetes* Lühe, *Derogetoides* Nicoll, *Genarchopsis* Ozaki, *Genolinea* Manter, *Genocerca* Manter, *Halipegus* Looss (Syns. *Vitellotrema* and *Genarchella*), *Hemipera* Nicoll, *Hemiperina* Manter, *Liopyge* Looss, *Ophiocorchis* Srivastava, *Progonus* Looss, and *Leurodera* Linton. In this paper the author describes a new trematode which is parasitic in the stomach of an Indian marine fish, and is referable to a new genus of the sub-family Derogetinae.

Genus—*Indoderogetes* Gen. nov.

*Indoderogetes purii*, n. sp.

Host—*Chirocentrus dorab* (Forsk.)

Habitat—Stomach

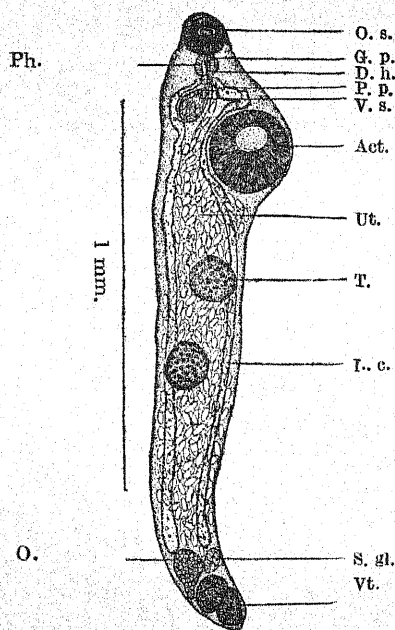
Locality—Puri, Bay of Bengal

Three mature specimens of this parasite were collected from the stomach of a marine fish, which was obtained from the Chilka lake on the coast of the Bay of Bengal. The worms are fairly muscular and light brown in colour. They have an elongated cylindrical body which is completely devoid of

\*The articles, 'A new parasite—*Stomachicola secundus*—of the subfamily Dinurinae Looss, 1907', 'Two new parasites of the genus *Aponurus* Looss, 1907 (Subfamily Lecithasterinae)' and 'A new parasite of the genus *Hysterolecitha* Linton, 1910', published in the *Ind. J. Vet. Sci. & Anim. Husb.*, Vol. IX, Part I, March 1939, constitute parts V, VI and VII respectively of this series. Through an unfortunate omission the general title and part numbers were not published.

scales and spines. The type specimen in permanent mount measures 1.6 mm. in length and 0.36 mm. in maximum breadth, which occurs across the level of the acetabulum. The suckers are fairly muscular, spherical structures, and are situated close together in the anterior third of the body. The oral sucker measures 0.4 mm. in diameter and is situated subterminally on the ventral surface, near the anterior end. The acetabulum is two-and-a-half times the size of the oral sucker and is situated at the end of the first quarter of the body. The oral sucker opens posteriorly into a spherical pharynx of 0.04 mm. diameter. The oesophagus is extremely small and bifurcates into two narrow, simple caeca which terminate blindly in front of the ovary.

The testes are two in number and are equal in size. They measure 0.12 mm. in diameter and are situated obliquely behind each other, separated by a distance of 0.1 mm. The posterior testis lies just behind the first half of the body-length. The vesicula seminalis is a small, flask-shaped sac which measures  $0.12 \times 0.06$  mm. in size. It is situated immediately behind the intestinal bifurcation and opens anteriorly into a small, narrow pars prostatica which is surrounded by prostate gland cells. The vesicula seminalis extends posteriorly to the level of the first third of the acetabulum. Terminally the pars prostatica joins the distal part of the uterus to form a small ductus hermaphroditicus. The latter opens on a small genital papilla which is situated in a median, shallow genital atrium close behind the oral sucker.



Ventral view of *Indoderogenes purii*

Act. = Acetabulum ; D. h. = Ductus hermaphroditicus ; G. p. = Genital pore ; I. c. = Intestinal caecum ; O. = Ovary ; O. s. = Oral sucker ; Ph. = Pharynx ; P. p. = Pars prostatica ; S. gl. = Shell gland ; T. = Testis ; Ut. = Uterus ; Vt. = Vitellaria ; V. s. = Vesicula seminalis.

The ovary is pear-shaped and measures  $0.12 \times 0.07$  mm. in size. It is situated in the space between the blind extremities of the intestinal caeca, the vitellaria and the hinder end. The shell gland mass of  $0.04 \times 0.03$  mm. size lies to the left of the ovary. The receptaculum seminis is absent. Laurer's canal is present. The vitellaria are composed of two oval bodies of  $0.06-0.07$  mm.  $\times$   $0.08-0.09$  mm. size, which are situated one behind the other at the extreme hinder end of the body. The uterus is well developed and pre-ovarian. It contains a very large number of yellowish brown, operculate eggs of  $0.03-0.032$  mm.  $\times$   $0.01-0.012$  mm. in size.

The excretory bladder is Y-shaped with its main stem bifurcating a little in front of the anterior testis into two cornua, which anastomose on the dorsal side of the oral sucker.

The new genus *Indoderogenes* differs from all the genera of the subfamily Derogenetinae, to which it obviously belongs, in the markedly anterior position of the acetabulum and the relative positions of the gonads, as well as in the length of the caeca and the pars prostatica.

#### GENERIC DIAGNOSIS

Body small, muscular, cylindrical and smooth. Suckers well developed situated close together in first third of body-length. Acetabulum, two-and-a-half times the size of the oral sucker, lies close behind the first quarter of body-length. Pharynx and oesophagus present; caeca terminate blindly in front of the ovary. Testes obliquely tandem, situated at about the middle of body. Vesicula seminalis flask-shaped, extending posteriorly up to the level of the anterior third of acetabulum. Pars prostatica and ducts hemaphroditicus small; genital pore situated on a small conical papilla close behind the oral sucker. Ovary in front of the two compact oval, vitelline bodies situated at the extreme hinder end of body. Receptaculum seminis absent. Laurer's canal present. Uterus pre-ovarian, eggs numerous, operculate, without filament. Excretory bladder Y-shaped, with the cornua anastomosing dorsal to the oral sucker. Parasitic in fishes. *Type species*—*I. purii*.

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# NEW ALLOCREADIDS (TREMATODA) FROM INDIAN MARINE FOOD FISHES

## PART V. A NEW PARASITE OF THE GENUS *LEPOCREADIOIDES* YAMAGUTI, 1936

BY

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(Received for publication on 14 June 1940)

(With one text-figure)

IN 1936, Yamaguti described a new parasite from a Japanese fish and created a new genus *Lepocreadioides* for its reception. The genus, with *L. zebrini* as the type species, was assigned to the subfamily Lepocreadiinae Odhner, 1905, of the family Allocreadiidae Stossich, 1903. In the following year, he added a second species from another Japanese fish to the genus. In this paper is described the third species of the genus. It is a common parasite infesting the gut of an Indian marine fish at Puri and Karachi.

### *Lepocreadioides indicum*, n. sp.

Host—*Platycephalus insidiator* Forsk

Habitat—Intestine

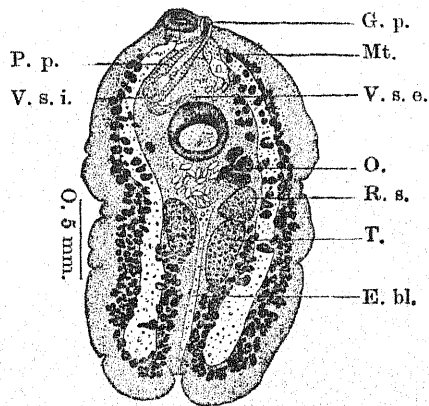
Locality—Puri and Karachi

A large number of specimens of this species was collected from the small intestine of *Platycephalus insidiator* examined at Puri and Karachi. The parasite has a foliate, ovoid body which is bluntly pointed anteriorly and broadly rounded at the posterior extremity. The lateral margins of the body are deeply crenated and the posterior end has a deep, median incision into which the excretory bladder opens. The worms in permanent mounts measure 1.0-2.32 mm. in length and 0.72-1.4 mm. in maximum breadth. Minute cuticular spines are present only in the pre-acetabular region, but small, deeply staining cutaneous cells are found uniformly distributed. They are specially prominent in the pre-acetabular region. The transversely oval, subterminal oral sucker measures 0.08-0.13 mm.  $\times$  0.09-0.19 mm. in size. The acetabulum is cup-shaped and measures 0.12-0.35 mm. in diameter. It is situated at the base of the anterior third of the body. The pre-pharynx is absent, but a pharynx is present and measures 0.06-0.08 mm.  $\times$  0.08-0.12 mm. The oesophagus is extremely small and is visible only in extended specimens. It bifurcates into two, simple, wide caeca, which terminate blindly a little in front of the hinder end.

The testes are two, longitudinal, elliptical bodies situated asymmetrically in the intercaecal space, one on either side of the excretory bladder. They lie close behind the anterior half of body. The anterior testis measuring 0.12-0.36 mm.  $\times$  0.08-0.16 mm. lies on the right side, while the posterior



testis of 0.14-0.4 mm.  $\times$  0.1-0.2 mm. lies on the left. The vesicula seminalis is divisible into two parts; a long, tubular, vesicula seminalis externa of 0.9-1.4 mm.  $\times$  0.3 mm. lying almost horizontally in front of the acetabulum and a small vesicula seminalis interna of 0.4-0.9 mm.  $\times$  0.4-0.5 mm. The club-shaped cirrus sac measures 0.4-0.6 mm.  $\times$  0.1-0.14 mm. in size and extends obliquely from the shallow genital atrium to the right in caecum, in level with the anterior border of the acetabulum. It encloses the vesicula seminalis interna, a small, well-differentiated pars prostatica of 0.04-0.15 mm.  $\times$  0.05-0.09 mm. surrounded by prostate gland cells, and a fairly long and simple ductus ejaculatorius, which opens proximally into a small cirrus. In some specimens, the pars prostatica is constricted into two parts. The genital atrium is situated to the left of the oral sucker level with its anterior border.



Ventral view of *Lepocreadioides indicum*, n. sp.

E. bl.=Excretory bladder; G. p.=Genital pore; Mt.=Metatrerm; O.=Ovary  
P. p.=Pars prostatica; R. s.=Receptaculum seminis; T.=Testis; V. s. e.=Vesicula seminalis externa; V. s. i.=Vesicula seminalis interna.

The ovary measures 0.1-0.24 mm.  $\times$  0.1-0.2 mm. in size and consists of three-pear shaped lobes, which join medially. It is situated on the left side just in front of the receptaculum seminis. The latter is a roughly triangular, thin-walled sac 0.22  $\times$  0.12-0.26 mm. in size, lying in front of the posterior testis. Laurer's canal is present. The shell gland mass lies in the space between the ovary, receptaculum seminis and the median line. The vitellaria are composed of small, irregular, follicles extending along the caeca from the level of the intestinal bifurcation to their blind ends, where they turn round and extend up to the level of the receptaculum seminis. The follicles overlap the caeca at several places. The yolk reservoir is a small, triangular sac, lying between the ovary and receptaculum seminis. The uterine coils occupy the median space between the acetabulum and testes. The eggs are not numerous and measure 0.057-0.068 mm.  $\times$  0.026-0.034 mm. in size.

The excretory bladder is a small, narrow tubular structure extending from the anterior border of the right testis to the hinder end.

*Lepocreadioides indicum*, n. sp. resembles the type species of the genus *L. zebrini* Yamaguti, 1936, in the general character of the body, in the digestive system, in the position of the receptaculum seminis, in the character of ovary and in the disposition of the vitellaria. It, however, differs from the type species in the character of the vesicula seminalis externa, in the extent of the cirrus sac, in the relative position of testes and in the more cephalad position of the genital atrium. The Indian species differs from the second species of the genus, *L. branchiostegi* Yamaguti, 1937, in the shape and size of its body, in the absence of spines in the post-acetabular region, in the shape of the cirrus sac and the vesicula seminalis externa and in the position of the receptaculum seminis.

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## SELECTED ARTICLES

### THE EFFICIENCY OF COPPER SULPHATE AND CARBON TETRACHLORIDE AGAINST *HAEMONCHUS CONTORTUS* IN ADULT SHEEP

BY

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#### INTRODUCTION

THE high degrees of anthelmintic efficiency of solutions of copper sulphate, copper sulphate with sodium arsenite, and carbon tetrachloride against *H. contortus* in young sheep have been demonstrated on a number of occasions [Seddon and Clunies Ross, 1929 ; Roberts, 1932 ; Clunies Ross and Gordon, 1934 ; and Gordon, 1935].

It has been shown that in young sheep, carbon tetrachloride possesses a very high degree of efficiency against adult *H. contortus*, and that treatment with this drug appears to be invariably satisfactory.

Copper sulphate solutions do not appear to be quite as efficient as carbon tetrachloride, and, moreover, are ineffective unless swallowed direct into the abomasum.

It has been shown by Clunies Ross [1934] and Mönnig and Quin [1935] that solutions of copper sulphate stimulate the closure of the oesophageal groove in sheep and tend to be swallowed into the abomasum in a considerable proportion of cases. The number of cases in which the drug is not swallowed into the abomasum has been noted to vary fairly considerably from one experiment to another. In 15 experiments carried out at this laboratory the following results were obtained :

Direct passage to abomasum : 184/209 cases or 88.0 per cent (variation 70 to 100 per cent).

Failure of direct passage to abomasum : 25/209 cases or 11.9 per cent (variation 30 to 0 per cent).

It thus appears that when using copper sulphate solutions as anthelmintics against *H. contortus*, one may expect treatment to be unsuccessful in as many as 30 per cent of animals, simply because the drug fails to pass directly into the abomasum. The average failure from this cause, however, should not be greater than about 12 per cent.

On the other hand, carbon tetrachloride appears to be efficient irrespective of its destination when swallowed. This is supported by recent experiments [Gordon], which showed that it possessed a high degree of efficiency against *H. contortus* when injected into the rumen through the abdominal wall.

The experiments described below were prompted by lack of experimental information on the efficiency of anthelmintics against *H. contortus* in adult sheep, and by recognition of the urgent need to control the worm burdens of such sheep. Although adult sheep frequently possess a varying degree of resistance to helminth parasites, they, particularly breeding ewes, nevertheless often become heavily infested. Adult sheep may harbour large numbers of helminths without showing obvious clinical evidence of parasitism, and thus may heavily contaminate pastures for lambs or other young sheep grazing with, or following, them. While treatment of adult sheep, therefore, may be necessary in order to cure them of helminthiasis, it is probably of greater importance in reducing the degree of contamination of pastures. It is now considered that a plan for control of helminthiasis of sheep, particularly haemonchosis, should begin with treatment of ewes before lambing, and the necessity for a highly efficient anthelmintic for adult sheep is therefore obvious.

Determination of a criterion for satisfactory efficiency presents a number of difficulties. Consider, for example, an adult sheep harbouring 8,000 *H. contortus*. A 70 per cent reduction will leave 2,400 worms, perhaps not sufficient to prevent recovery of the sheep, but assuming that half of them are females (which produce up to 5,000 eggs each per day), the sheep may still distribute 6,000,000 ( $1,200 \times 5,000$ ) eggs every 24 hours. With a 90 per cent efficiency, 800 worms would remain, and the 400 females would produce 2,000,000 eggs every 24 hours. It thus appears that while treatment may be effective in curing helminthiasis, its effect in reducing infestation of pastures is not necessarily as real. In other words, a moderately satisfactory treatment may prevent deaths from haemonchosis, but may not prevent development of heavy infestation in lambs pastured with ewes. A highly efficient treatment is necessary for this latter purpose. Owing to variations in degree of efficiency, it was finally decided that a treatment which reduced the egg count by 70 per cent or more should be considered satisfactory.

#### EXPERIMENTAL PROCEDURE

The majority of sheep used were artificially infected by dosing with larvae and were maintained in concrete pens throughout the experimental period. A few sheep which had become infected by grazing on infested pastures were also used. Egg counts were carried out daily for at least 10 days before and 14 days after treatment. A decrease of 70 per cent or more in egg count was considered to indicate that treatment was satisfactory. In many cases the decrease in egg count was in the vicinity of 90 per cent. Details of egg counts are not given owing to considerations of space.

#### Dosage

(a) 1 fl. oz. of 4 per cent solution of copper sulphate.—This is the dose rate usually prescribed for adult sheep in Australia. Alternative dose rates are 2 fl. oz. of 2 per cent solution or 20 ml. of 5 per cent solution. Preliminary

tests indicated that 1 fl. oz. of 4 per cent solution was an unsatisfactory treatment.

(b) 2 fl. oz. of 4 per cent solution of copper sulphate.—This dose rate, double that usually prescribed, was tested as soon as it was evident that the usual dose was unsatisfactory. The double dose did not appear to possess undesirable toxic properties apart from causing temporary loss of appetite. The ordinary dose also has this effect in some individuals.

(c) 2 ml. carbon tetrachloride in 3 ml. liquid paraffin.—This is the dose widely used, for sheep six months old and over, against *Fasciola hepatica* and *Haemonchus contortus*.

### Results

Results of the treatments are summarized in Table I.

TABLE I

Efficiency	1 fl. oz. 3 per cent CuSO <sub>4</sub> solution		2 fl. oz. 4 per cent CuSO <sub>4</sub> solution		2 ml. CCl <sub>4</sub> in 3 ml. liquid paraffin	
	No.	Per cent	No.	Per cent	No.	Per cent
Satisfactory . . .	6/21	28.5	22/24	91.6	9/10	90
Not satisfactory . .	15/21	71.4	2/24	9.3	1/10	10

It is seen that 1 oz. of 4 per cent solution of copper sulphate was an unsatisfactory treatment against *Haemonchus contortus*, and that 2 oz. of 4 per cent copper sulphate solution or 2 ml. carbon tetrachloride in 3 ml. liquid paraffin were very satisfactory.

### DISCUSSION

The purpose of this note is to record the observation that, while the usually prescribed dose of carbon tetrachloride is effective against mature *H. contortus* in adult sheep, copper sulphate solution, in the dose usually prescribed for adult sheep, is not effective. It was found, however, that twice the usual dose, namely 2 oz. (60 ml.) of a 4 per cent solution was highly efficient. (If it is considered that 2 oz. is too bulky a dose, 1 oz. of an 8 per cent solution may be used).

Copper sulphate solution is widely used on account of its cheapness and comparatively wide safety margin. Nevertheless, despite the above findings in regard to the efficient dose rate for adult sheep, the dose rate for younger sheep, up to 18 months of age, is effective and should not be exceeded.



At the earliest opportunity trials will be undertaken to determine the efficient dose rate of the copper sulphate nicotine sulphate mixture for haemonchosis in adult sheep, since this mixture has the additional advantage of being relatively effective against the immature forms of *H. contortus* and against *Trichostrongylus* spp.

#### CONCLUSIONS

1. The usually prescribed dose of copper sulphate (1 fl. oz. of 4 per cent solution) is not an efficient treatment against *H. contortus* in adult sheep.
2. A dose of 2 fl. oz. of 4 per cent solution of copper sulphate (double the usually prescribed dose) is very effective against *H. contortus* in adult sheep.
3. A dose of 2 ml. carbon tetrachloride in 3 ml. liquid paraffin is very effective against *H. contortus* in adult sheep.

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# THE TREATMENT OF BABESIELLOSIS (*B. ARGENTINA*) WITH ACAPRIN

BY

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(Reprinted from the *Australian Veterinary Journal*, Vol. XV, No. 3, June 1939)

## INTRODUCTION

ACAPRIN, which is described as  $N_1N^1$ —(bismethylquinolylmethyl-sulphate-6—carbamide (Bayer), is a synthetic drug now widely used as a specific against piroplasmosis in domestic animals in various parts of the world.

It is usually employed as a solution of 5 per cent and has many advantages because the dose employed is relatively small, the solution is stable, and because it can be used subcutaneously its application is quite easy.

Legg [1936] previously reported on its use in the treatment of piroplasmosis (*P. bigeminum*) in cattle in Queensland. He found it very effective when employed intravenously, but had the disadvantage that when so applied it sterilized the animal. So far there has been no publication in Australia dealing with the use of this drug against babesiellosis (*B. argentina*). This particular form of tick-fever is now known to be by far the most common type in this country, although piroplasmosis and anaplasmosis (*A. marginale*) are both known. The organism responsible appears to be identical with that described by Lignieres in 1903 in the Argentine and also with the one seen by Sohns in the Dutch East Indies in 1918. If not identical with, it is at least closely related to *B. berbera* found in N. Africa by Sergent in 1924.

## ANIMALS TREATED

Among a number of cattle treated both in the field and under experimental conditions the following are selected for description.

### (a) *Non-splenectomized*

*Bovine 310*.—Steer one-and-a-half years old, good condition.

This animal was an anaplasma carrier (*A. centrale*) and was inoculated on 7th March 1938, with 20 ml. citrated blood ex-bovine 321, a babesiella carrier. At the same time it received 20 ml. citrated blood intravenously ex-370, a carrier of *P. bigeminum*. Piroplasms were seen on the 4th, 5th and 6th days after inoculation, but the reaction was mild and called for no treatment. Babesiellas appeared in the blood on the 12th day, but were difficult to detect (evening temperature  $105.6^{\circ}$ ). They were frequently seen in smears taken on the morning of the 13th day (temperature  $106.4^{\circ}$ ),

when 6 ml. acaprin were given. Within a few hours the temperature had fallen to  $104.4^{\circ}$  and parasites had disappeared. Recovery was uneventful.

*Bovine 379.*—Steer one year old, good condition.

Inoculated subcutaneously with 20 ml. citrated blood on 25th March 1938, ex-321, a babesiella carrier. The temperature was normal for 13 days during which blood smears showed no parasites. On the evening of the 14th day the temperature was  $104^{\circ}$  and the blood still negative; 15th day  $106^{\circ}$  (morning) when parasites were noted for the first time. The temperature remained around  $106^{\circ}$  until the afternoon of the 16th day, parasites steadily increasing in the peripheral blood. At this stage 6 ml. of acaprin were given.

The following morning (17th day) the temperature had fallen to  $101.2^{\circ}$  and parasites were rare. A few hours later they disappeared completely. Recovery was uneventful.

*Bovine 381.*—Steer one year old, good condition.

The animal previously referred to (379) was bled on the 16th day before the acaprin was injected and 5 ml. citrated blood inoculated subcutaneously into 381. On the fourth day the temperature was between  $105^{\circ}$  and  $106^{\circ}$ , but the blood was negative. The following day (fifth) the temperature fluctuated between  $104.5^{\circ}$  and  $105^{\circ}$  and parasites appeared. During the sixth day they steadily increased in number, and in the afternoon at 3 P.M. when acaprin was given (6 ml.) the temperature was  $106.8^{\circ}$  and parasites were common. A few parasites were seen on the following morning (seventh day), the temperature being  $105.2^{\circ}$ . No further parasites were subsequently seen and the temperature rapidly declined to normal.

*Bovine 487.*—Stud Shorthorn bull, one-and-a-half years old, prime condition.

Inoculated on 19th April, 1939, with 20 ml. citrated blood ex-bovine 480, a carrier of both *P. bigeminum* and *B. argentina*. Temperature and blood examinations were normal for 12 days. On the 13th, 14th and 15th days there was some fluctuation in temperature which eventually reached  $106.2^{\circ}$  on the evening of the 16th day when babesiellas appeared. The following morning (17th day) both piroplasms and babesiellas were present in considerable numbers (temperature  $105.2^{\circ}$ ) when 6 ml. acaprin were given. Piroplasms had disappeared by the evening, but a few babesiellas were still present. The next day (18th) the temperature was down to  $102.4^{\circ}$  and the blood negative. Recovery was rapid.

#### (b) Splenectomized animals

In view of the successful use of the drug in ordinary animals it was decided to extend its application to the treatment of animals from which the spleen had been removed. From previous observations made it was known that the disease ran a rapid and fatal course in animals so treated.

*Bovine 397.*—Steer 2 years old, good condition, a carrier of *A. centrale* (South African origin).

Inoculated subcutaneously 2nd August, 1938, with 20 ml. citrated blood ex-381, a babesiella carrier. Splenectomy performed three days afterwards. The temperature fluctuated during the three or four days following splenectomy and reached  $105^{\circ}$ , but soon settled to normal. On the evening of the

14th day it was again up to  $105^{\circ}$ , but the blood was negative. On the morning of the 15th day it was over  $105^{\circ}$  and parasites appeared in the blood. These steadily increased in numbers and during the afternoon a full dose of acaprin (6 ml.) was given. On the 16th day the temperature was still over  $105^{\circ}$  and a few parasites present. A further dose of acaprin (6 ml.) was given in the afternoon.

The temperature had fallen to  $102.8^{\circ}$  by the morning of the 17th day, parasites had disappeared, and from then on recovery was uneventful.

*Bovine 441.*—Steer 2 years old, good condition.

Inoculated extravenously with 100 ml. citrated blood ex-376, a babesiella carrier, on 29th October, 1938. Splenectomy performed five days later. The temperature steadily rose during the four to five days after splenectomy and reached  $105.6^{\circ}$  on the evening of the 11th day, when a few babesiellas appeared in the blood. The next day (12th) they were still rare, but the temperature was now between  $106^{\circ}$  to  $107^{\circ}$ , so 6 ml. acaprin were given at 10 A.M.

By the morning of the 13th day the temperature was down to  $102.4^{\circ}$  and parasites had disappeared. Recovery rapidly followed.

*Bovine 442.*—Steer 2 years old, good condition.

Inoculated intravenously with 100 ml. citrated blood ex-377, a babesiella carrier, on 29th October, 1938. Splenectomy performed 5 days later.

After splenectomy the temperature steadily rose for four days and on the evening of the ninth day was  $106.8^{\circ}$ . Blood was negative. On the 10th day parasites first appeared but were rare. The temperature still remained over  $106^{\circ}$  and acaprin was given (6 ml.) in the evening. The following morning (11th day) it had fallen to  $104^{\circ}$  and parasites were still seen, but were rare. In a few hours, however, they had disappeared and the temperature had receded further. Recovery was rapid.

In all the cases described the acaprin was given subcutaneously.

#### CONCLUSIONS

Acaprin in doses of 6 ml. subcutaneously applied to cattle reacting acutely to *B. argentina* is followed by a rapid disappearance of the parasites from the peripheral blood and a fall in temperature. Animals so treated rapidly recover.

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## FERTILE MARE MULES\*

BY

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(Reprinted from the *Journal of Heredity*, Vol. 30, No. 12, December, 1939)

(With Plate III)

THERE are very few living animals whose mothers are mules. Two of these rarities are owned by the Texas A. and M. College, and another is owned by W. H. Mobley and Son of Columbus, Indiana.

One of the two in Texas is a mare mule by a jack out of 'Old Bec' described in this Journal, Sept., 1928. She is not a three-quarter ass as her ancestry would indicate but a mule in all her features. Unlike her mother, she proved to be sterile. The other Texas animal is the son of a saddle stallion out of 'Old Bec'. He is a five-gaited saddle horse and has been shown as such at Texas fairs. He has none of the ass-like qualities of his mule mother. He has been mated to several mares and the foals by him show no evidence of hybrid blood.

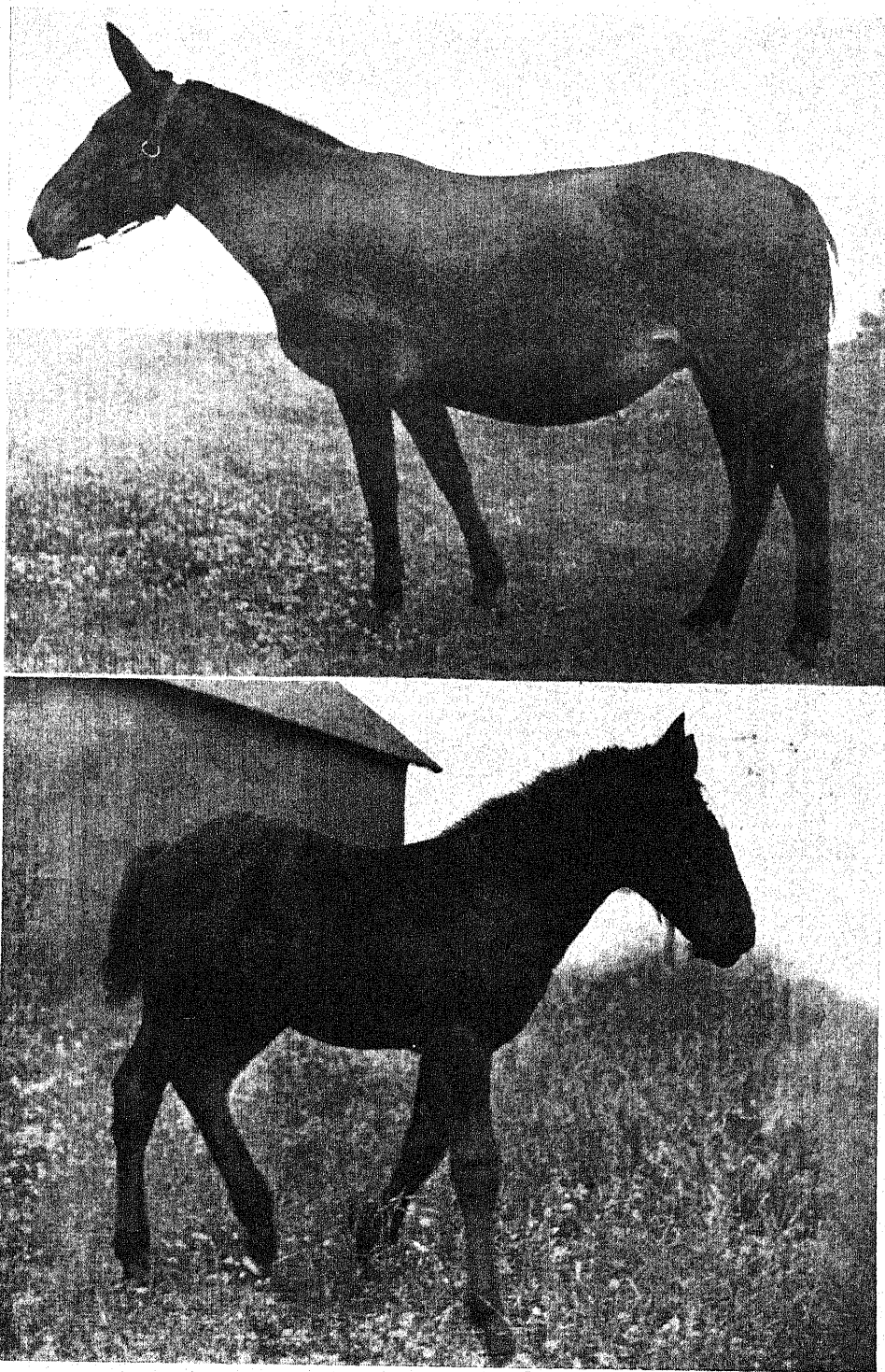
Another animal with a mule mother, is a male foal by a Percheron stallion and was five weeks old in June, 1938, when photographed (Plate III). He exhibits the Percheron characteristics for a foal of that age. There is complete absence of any influence of the ass ancestry coming to him from his mother. His owners intend to keep him and test him by breeding.

These three living animals explain those rare occasions when a female mule becomes a mother. It is known that the ass has a greater number of chromosomes than the horse. This difference does not interfere with an ovum of a mare developing when fertilized by a sperm of a jack; but when the hybrid reaches physical maturity and the gonads begin to function the inequality of the chromosome numbers prevents the reproductive glands from producing sex cells. The author has examined glands of male mules after castration but has never found any spermatozoa.

The production of the three animals by mare mules indicates that their ovaries did mature viable ova. Since two of the foals by stallions are horses without any features of the mule or ass, it is certain the ova that produced them carried no chromosomes from the ass. The mare mule out of 'Old Bec' and by a jack is in every way a mule; not an animal with three-fourths of her genes from the ass. In this case the ovum evidently carried no chromosomes of the ass but only the chromosomes which its mother received from her mother, a female horse. The explanation appears to be, that if the female mule produces an ovum without ass chromosomes it is viable. It could happen, if in the division of the oocyte, all the ass chromosomes clung together and went into the polar, or waste cell, the final ovum would contain only the

\* A few days after this note was received similar information and another illustration of the Indiana mule and her colt were submitted by Dr. Edwin E. Jacobs, Ashland College, Ashland, Ohio.





A fertile mule and her colt

Photographs of the female mule from Indiana and the horse-like foal she produced when mated to a Percheron stallion. The colt was five weeks old when the picture was taken. This survey of three instances of fertile mules suggests that some female mules may occasionally produce a pure horse ovum, uncontaminated by ass ancestry, and that these rare ova are fertile.



chromosomes received from the horse ancestry. If this hypothetical explanation be true then a viable ovum of a mare mule would carry the haploid number of horse chromosomes just as the ova of all female horses. On the other hand no viable ova are produced by female mules except the rare ones in which the sister polar cell carried away all the ass chromosomes.

This would explain the bearing of a horse-like colt by a fertile mule, recorded skeptically by Lloyd Jones in this Journal in 1916. It was reasonably held that an animal with one-fourth ass ancestry should have some characters of the ass, but as pointed out above, this instead of being ground for doubt, may really be what is to be expected in cases of mule fertility. As the word of the breeders was the main evidence, the rare occurrences were frequently looked upon with more doubt than interest.

Further evidence that the foregoing hypothesis is true is found in a recent statement from the Texas A. and M. College that "the son of the 'Old Bec' is fertile and has quite a number of colts around that section of the country. His daughters also are fertile. He is rather a popular sire locally and is used for saddle purposes". None of his offspring show any reversion to their ass ancestry. If he carried any of the chromosomes of the ass, hybrid qualities would appear in his offspring. The absence of ass-like qualities seems conclusive evidence that he himself originated from an ovum with no chromosomes from his grandsire, the jack.

In 1918 the Kentucky Experiment Station purchased a mare mule that had aborted a fetus of about four or five months of age. She was mated with a stallion but failed to conceive. The fetus was preserved and appears mule-like. The high fetal mortality rate of mules that conceive may perhaps be explained by combinations of chromosomes being formed which are only partially viable.

The dream of mule breeders has been to find fertile mare mules, hoping to get a race of perpetuating hybrids. The dream it seems cannot be realized as the reversion to the horse of the hybrid ancestry takes place, when they are fertile.

If the above hypothesis is correct, the two male horses out of mare mules hereditarily have no maternal grandfathers as the hereditary material which each mother mule received from her sire was all eliminated in the normal random assortment as the ova were produced, and only the mother's material gift of chromosomes entered the viable eggs from which the two horses evolved.

## ABSTRACTS

A note on the inheritance of kemp in black-face sheep. D. M. BRYANT (1933). *J. Textile Inst.* 24, T 309

THE term 'kemp' is applied to the opaque fibres, occurring in the outer coat of the primitive sheep. It is much coarser than the other fibres and, owing to the presence of an air-filled medulla, has a dead white appearance. The fibre is very brittle, possesses a marked wave in one plane only and very low tensile strength. The presence of these fibres in wool is a matter of deep concern both to the breeder and the trade. To the breeder it means depreciation in the value of the fleece and as for the manufacturer of woollen materials he can utilise the low quality wool for coarsest fabrics only. No method of removing the kemp is available except the laborious one of hand-picking. Fortunately the fine woolled sheep are mostly free from kemp but in some of the other breeds, e.g. Cheviot, it is responsible for considerable wastage. Investigations were undertaken by the author to ascertain the possible mode of inheritance of kemp and the breed chosen for this purpose was the Scottish Mountain Blackface. The experimental sheep were kept under standard commercial conditions from year to year, which, obviously, could not be controlled.

The experimental evidence has shown that there is a hereditary basis for the expression of kemp in the fleece and that it is influenced to some extent by the system of feeding. Like other wool fibres, kemp also responds in thickness and rate of growth in length to altered conditions of feeding, and due to its greater original thickness and marked conspicuousness in the fleece, better nutrition leads to apparently greater increase in the amount of kemp visible in the fleece on the living sheep. Thus these results lead to the conclusion that if an endeavour is to be made to reduce the amount of kemp in wool, attention should be devoted primarily to the manipulation of the breed constitution rather than the environment of the sheep.

In the present investigation the analysis of samples from parental and filial generations has shown that kempiness in the fleece is inherited, that rams with fleece containing less than 1 per cent of kemp leave greater numbers of progeny which approximate to this figure than do more kempy rams and, lastly, that it is possible to produce a flock with low kemp content by using rams of less than 1 per cent of kemp and eliminating the kempy sheep. [H. B. S.]

The improvement of Chinese and other carpet wools. R. H. BURNS A. JOHNSTON and W. C. CHEN (1940). *J. Textile Inst.* 31, T 37

IN this article, the authors have formulated a tentative guide of wool type for carpet wool producers. The characteristics and origin of some of the different types of carpet wools have been described and opinions of the trade in respect of the relative values of different samples of desirable and undesirable carpet wools have been recorded. It is common knowledge that carpet wools are obtained from sheep reared under primitive conditions in various parts of the world. With the exception of a few thin-tailed varieties, most of this wool comes from the fat-tailed and broad-tailed varieties of sheep in Asiatic countries. The type of fleece in these sheep is composed of a mixture consisting of a long hairy outer coat, a finer undercoat and true wool and a third type of fibre, the so-called 'kemp'. The outer coat consists of *heterotypical fibres*, i.e. hair and intermediate fibres. As the name implies, these fibres are neither wool nor kemp. Originally this designation was confined to those fibres which within the same shaft showed characteristics of wool fibres, kemps and hairs. Now it is restricted to those fibre shafts which possess the characteristics of both wool and hair in the same fibre, particularly in respect of medullation and non-medullation. *True wool* consists of fibres which form the fine undercoat of the mixed-wool sheep. *Kemps* are the brittle fibres which lie loose in the fleece. These are not normally shed. In the present investigation the authors have analysed both microscopically and macroscopically seven samples of carpet wools and a sample of Romney wool for fibre types, fibre thickness and fibre length. They have observed that the Vicanere and Aleppo wools are considered the best carpet wools in the world by carpet



manufacturers and the Thibetan is supposed to be the best among the Chinese wools. The Vicianere wool is produced in north Central India. It is soft, lively and springy and these are the characteristics which are highly valued in carpet wools. The Aleppo wool is grown in Syria, in Asia Minor, and possesses good length and is well known for its colour, strength and resiliency. The Woozie wools, which are produced in the neighbourhood of Shanghai, are regarded by the trade as one of the poorest of carpet wools. The macroscopical analysis of carpet wools has indicated that the more desirable types according to manufacturers contain lower percentage of true wool and kemp and larger percentage of heterotypical fibres. The Vicianere and Aleppo wools on examination admirably satisfied this requirement. The authors conclude that Chinese wools are not as good as the Vicianere and Aleppo wools in the macroscopical characteristics. The Romney wool sample showed only true wool fibres. It is observed that an ideal carpet wool should contain :—

- (i) at least 15 per cent by count or 35 per cent by weight of heterotypical fibres. These fibres should have an average thickness of at least 30 microns and the fibre sizes should not vary more than 15 per cent. The average lengths of these fibres should be at least 100 mm. for normal growth (12 months), and the variability of the fibre length should be less than 20 per cent ;
- (ii) not more than 2 per cent by count or 4 per cent by weight of kemp fibres. The dimensional characteristics of the kemp fibres are not so important. The important thing is to eliminate kemp from the fleeces and work on the subject has shown that this can be done by paying attention to the selection of breeding rams and ewes ;
- (iii) not more than 85 per cent by count or 65 per cent by weight of true wool fibres. These fibres should have an average thickness not exceeding 25·4 microns, and their variation in fibre thickness should not exceed 25 per cent. They should have an average length of at least 100 mm. for normal growth (12 months) and the variation in fibre length should not exceed 25 per cent.

[H. B. S.]

**Present day problems in animal nutrition.** ISTVAN MOSKOVITS. *Monthly Bulletin of Agricultural Science and Practice, International Institute of Agriculture, Rome—April 1940. No. 4*

AT the instance of the General Assembly of the International Institute of Agriculture it is proposed to deal in the above monthly bulletin, in a series of articles, with the most striking investigations and practical trials in the field of animal nutrition and to review the outstanding results achieved during recent years. The first of this series relates to 'Self-sufficiency in fodder supplies' or, in other words, the tendency in various countries, notably in Europe, to utilise home-grown fodder for livestock. Naturally, therefore, this trend, which is a reaction to the tendency of obtaining maximum yields by using suitable feeding stuffs irrespective of any regard for their origin, has led to much radical alteration in fodder production and dietary methods. The present study discusses the different methods adopted for attaining this objective, i.e. the rearing of livestock on home-grown feeding stuffs without in any way adversely affecting the amount or quality of animal products but rather, on the contrary, aiming at the maintenance of a steady increase.

Imports consist mainly of concentrates, i.e. products of high nutritive value in proportion to their weight. The feeding value thus justifies the cost of transport which is often heavy. Intensification of the output of home-grown produce would, in many countries, result only in increased quantity of fodder crops. Most fodders produced on the farms, however, are bulky, i.e. contain less nourishment and a large proportion of roughage. Ruminants can utilise roughage better than other classes of livestock whose ration requires a higher concentration of essential nutrients. The question of basing the ration of ruminants mostly or totally on home-grown produce does not, therefore, present insuperable difficulties. However, in this connection, it is observed that though from the technical point 'the supply of carbohydrates presents no difficulty in countries which have adopted a self-sufficiency policy, the same cannot be said as



regards proteins. The large quantities of protein necessary to increase the milk yield cannot be obtained by adding hay, silage, cereals bran to a basic ration drawn from the fodder crops produced on the farm, without making it impossible for the animal to absorb the whole ration because of its high dry matter content.' On the basis of the available results of investigation in different countries, it is concluded that 'when the diet is limited to fodders produced on the farm, the hope of obtaining maximum yields must be abandoned.' Therefore, when selecting animals for breeding purposes, preference should be given to those maintaining a good average yield on the available fodder rather than high yielders.

In regard to measures for increasing the fodder output, the generally recognised methods are enumerated, viz. (a) extension of cultivated areas, (b) increase in yield and more rational use of meadows and grazing lands, (c) increase in fodder crops. It is stressed that 'it is the manner of using the meadows which determines the protein content and the influence of every other factor is of less importance, being almost always indirect'. Under the self-sufficiency system adopted by several countries, the meadows and grazing lands acquire all the more importance in that they supply the greater part of the necessary protein. With regard to obtaining increase in fodder crops, it is observed that 'by a rotational selection of fodder plants, by encouraging the cultivation of those giving the best yields or by reducing the fallow periods of arable land (inter-row cultivation and catch crop), it is possible either to increase the quantity of fodder harvested over the same area or to reduce the area cultivated while maintaining production at the same level'. As regards the plants to which preference should be given it is suggested that those producing the greatest quantity of nutritive substance over an equal area are to be preferred. It is stated that a plant of capital importance has been found in the sweet lupin, especially when grown in sandy soil and the green fodder produced from it is much liked. Repeated feeding trials with dairy cattle, sheep, horses, pigs and poultry show that sweet lupin seeds are pleasant to taste, easily digested and that their effects correspond to their theoretical nutritive value. It has been observed that in feeding pigs and poultry, it is necessary to supplement the protein content of the lupin seeds with albuminoids of animal origin in order to improve its biological value. All kinds of animals like the green fodder obtained from the sweet lupin and it does not become woody till very old. Another fodder plant of recognised value, the cultivation of which is steadily increasing, is the marrow kale (*Brassica oleracea acephala*). The green material contains an average of 2.0 per cent of digestible crude protein, 1.5 per cent of net digestible protein and its starch equivalent is 8.0 kg. Feeding trials have shown that considerable quantity of protein can be economised by feeding this plant to dairy cows. Considerable attention is also being paid to catch crops. An effort is being made through these crops to increase the output of feeding stuffs and consequently the quantity of vegetable protein also; at the same time most of the silage is made from these crops. The most important forage plants grown as catch according to the kind of soil, climate and season at which the sowings are made, are the following: red clover, several other varieties of clover and grasses, sweet lupin, serradelle, forage cabbage, mustard, rye, colza, turnip, rape, green maize, fodder mallow, etc. The commonest mixtures are grass and red clover; rye and vetch; oats, barley, vetch and peas; beans, peas and vetch.

Certain waste and by-products have always played an important part in the feeding of animals. Innovations such as the 'Anti-Wash Campaign', i.e. collection and utilisation of household rubbish have led to the accumulation of large quantities of complimentary feeding stuff for animals. This is mostly used for pig feeding. The use of meat meal and powdered blood is not a novelty in itself but considerable progress has been made in regard to the inexpensive collection and processing of these products.

Much work has been done on the collection and preservation of beet leaves which are a valuable feeding stuff for livestock on account of their protein content and relatively less proportion of roughage. With the modern technique of artificial drying the loss of nutritive value has been reduced to 25 per cent of digestible crude protein and 10 per cent of its starch equivalent. Efforts have also been made to extract protein from the juice which is generally lost in the process of preparing potato starch and several dry products of proved nutritive value have been obtained for feeding sheep and pigs. Increased cultivation of linseed has led to the discovery of a valuable fodder in the husks of the seed. It has been shown that the husk contains 5.3 per cent of digestible

protein and a starch equivalent of 30.6 kg. Among the dairy by-products, whey has received considerable attention. Attempts are being made to discover a method of preserving this product with a view to make available a concentrated food rich in protein. Nutrition tests based on dried whey have shown that its content of nutritive principles corresponds as a rule to that of forage barley, while its mineral content, chiefly lime, is higher than that of barley. It can be given freely to pigs, young cattle and grown calves but the somewhat sour taste sets a limit to the quantity which can be added to a ration. Poultry were found to refuse dried whey after a few days, probably because of the high proportion of lactose (45 to 50 per cent) and lack of specific ferments (lactase). Nutrition tests have shown that the use of neutralised whey has no bad effect on the health of the animals if delivered daily when fresh in wooden containers.

A passing reference has been made in regard to investigation on substitutes, i.e. substances which are likely to fill in the shortage of concentrates and especially of foods with a high protein content. The cultivation of yeasts by sacrificing carbohydrates and inorganic nitrogenous salts, the substitution of protein with non-albuminoid nitrogenous substances and recent researches in Germany on the utilisation of wood sugar for livestock feeding have been described. Under the self-sufficiency system the question of suitable preservation of fodder with the least loss of nutritive principles is of paramount importance. In this connection, it is observed that the use of hay cocks in hay making is now widespread and in many countries subsidies are granted by Government to enable the farmers to purchase them. The most recent models are made of wire on which the newly mown fodder is hung out to dry. The production of silage has been gradually improved during the past few years by processes such as the addition of different acids, of fermentable carbohydrates, lactic ferments, by means of partial wilting of green fodders, etc. Large Government subsidies have been granted for the construction of silos and this shows the importance attached to ensilage methods. Greater attention is also now being paid to artificial drying of fodder crops. The cost involved appears to be fairly high but the product has a high nutritive value and can be given to any class of livestock, though particularly useful for feeding milch stock.

The above resume will show that during the last few years, the self-sufficiency movement has made rapid strides in European countries and the numerous problems confronting it are receiving concerted attention. Attempts are being made to regulate the time of calving, lactation periods, etc., in accordance with the availability of fodders during different seasons and the present trend does not aim at maximum yield, which is obtainable only with the aid of foreign concentrates, but good, average yields from strong healthy stock suited to thrive on the bulkier home-grown fodders and available vegetable proteins.

In regard to this self-sufficiency problem from the Indian point of view, it is interesting to record that the most recent estimates of the available fodder resources (cultivated) in this country are as follows:—

(a) Straw of crops (moisture 10 per cent)	135,199,082 tons.
(b) Dry matter content of above	121,679,174 tons.
(c) Green fodder (moisture 80 per cent)	169,056,479 tons.
(d) Dry matter content of above	33,811,296 tons.

The amount of concentrate expressed on dry matter basis is cakes <i>plus</i> cotton seed	3,829,746 tons.
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On the basis of 215 million heads of bovines (as we have an available supply of 155,490,470 tons of dry fodder from cultivated sources) it is found that only about 4.4 lb. of dry fodder per head per day are obtainable. Even if bran, pollard, etc. are considered to be fodder and the amount available is added to the amount of coarse fodder, this will mean only an addition of a little over an ounce per head per day. In this calculation, sheep, goats and equines are not taken account of. If one assumes an average body-weight of the Indian animal to be 400 lb., then the food requirement would be about 8 lb. of dry matter per day (inclusive of the concentrate). For hard working animals and milch cows the amount will have to be increased. The amount of concentrate (cakes and cotton seed) and bran, etc. available works out to be about 0.2 lb. per head per day. As the fodder supply should provide about 8 lb. of dry matter per day and since only 4.4 lb. are available from cultivated sources, there is a shortage of 45 per cent of this substance.

It is obvious that grass and hay will tend to rectify the deficiency. Whether we can get the additional 45 per cent of the requirement from fodder grasses cannot be accurately estimated. Much more than this is certainly potentially available and it is for this reason that grass land improvement measures and better utilisation of forest areas are of so much importance. [H. B. S.]

**The theory of chromosomes. Discussion of unlike twin brothers.** A. LEWIS  
OSWALD (1940). *Jersey Bul.* 59, 773.

**A**BREEDING establishment does not exist on possession of a bull. One soon notes that great bulls have brothers of mediocre or poor quality, even when bred to the same cows. This is observed in the human family where full brothers vary to a great extent in the success they attain in life. One may celebrate prematurely on bringing together a bull with a good pedigree with cows having good pedigrees. Inquiry explains how this is possible.

Each cow or bull has 32 'atoms of the flesh' or chromosomes, of which 16 came from its mother and 16 from its father. These numbers never vary. No one, however, has been able to explain what determines which 16 of the 32 are passed on to the progeny by the parent. Do they struggle among themselves for this privilege? In passing 16 of the 32 chromosomes to the progeny there is no loss to the parent, at least there is no evidence to the contrary; they are replaced immediately by 16 identical ones.

Each chromosome has one vote for a particular character, brown eyes or blue eyes for example. If the chromosomes for this character from each of the parents vote for one colour, that colour wins; if they vote for different colours, the stronger or dominant one wins. Brothers may have eyes of different colours for this reason. Likewise, bulls which are full brothers may sire progeny that are greatly different. If two full brothers have received identical chromosomes from each parent they will be identical. The mathematical possibility of this ever occurring is, however, quite remote. It is thereby possible for twins to be as similar or as dissimilar as full brothers born at different times. All brothers and sisters have many characters in common, very rarely are they identical.

Each bull has 32 chromosomes and passes 16 to his progeny. It is probable that he passes a different combination of 16 each time. Bulls which transmit, in some unknown manner, almost the same combination of 16 chromosomes each time have progeny that are 'as similar as the proverbial peas in the pod'. A sire, therefore, which has been well bred for generations will be most apt to produce good calves. A bull whose one parent was good and the other bad will give about 50 per cent good calves and 50 per cent poor ones.

'The cow is important, but the bull is the old boy that counts. He is the "papa" of the whole crop of calves, while each "mamma" has just her own child'. On the other hand a dairyman breeds for cows; he has no need for a barn full of bulls. The breeding of cows which produce large quantities of good quality milk and reproduce their kind is the goal of the dairyman.

(NOTE.—The writer considers cattle to have 16 pairs of chromosomes. In his book entitled *Animal Breeding Plans* (Collegiate Press, Ames, Iowa, U. S. A.) J. L. Lush gives a summary of recent reports of chromosomes in mammals and poultry. Here one finds that three different ideas exist as to the chromosome number in cattle: (1) 30 pairs according to Krallinger, 1931; (2) 19 pairs according to Wodsdalek, 1920; and (3) 17 pairs according to Masui, 1919. This paper further considers both the male and female of the bovine species homogametic, whereas Lush reports that in practically every species of mammal the male is heterogametic or has an *uneven* number of chromosomes; the female mammal is homogametic or has an *even* number of chromosomes.

The paper does, however, give the general idea of genetic constitution of an individual and does so in terms which are easily understood. The information, if properly interpreted, should be very helpful to the animal breeder.) [J. N. W.]

**Color genes in Holstein-Friesian by Brown Swiss crosses.** RALPH J. BUSHNELL  
(1940). *J. Hered.* 31, 253.

**B**BROWN SWISS males were crossed with Holstein-Friesian females. The data obtained add to the probable gene constitution of the Brown Swiss breed and illustrate the result of crossing white-faced Holstein-Friesians with a self-coloured breed. F<sub>1</sub> and back cross data are used.

Genes of interest in this study are : ' **B**, causing black hair, which is modified from the solid color by the presence of the recessive gene **s** which causes white spotting of the coat ; **bs**, or lack of the factor for " blackish " hairs as found in the Brown Swiss and Jerseys ; **d**, or lack of the dominant diluting factor which effects black ; **l** the normal allele of the recessive dilution factor **i** ; lack of the **W** factor which causes whitening in the muzzle, coat, etc ; and **In**, or dominant white spotting in the inguinal region.' Some Holsteins also have a dominant gene for the white star (**Ws**)

The  $F_1$  data of this study show that the Brown Swiss carry '**bb**, which would result in red, except for the presence of **Es** (partially epistatic to red) and '**ii**, the latter being mostly effective on dilution of red ; **D**, which dilutes black to dun (one Brown Swiss male was apparently heterozygous and the other homozygous for **D**) ; **W**,—(with one exception, the  $F_1$  animals did not show the effects of the whitening gene because, probably, black is epistatic to it) ; **inin**, or lack of all white spotting in the inguinal region—'.

This cross gives an issue which is complicated because of the presence of **Bs**. Brown Swiss are self-coloured, **S**, and they may carry **i**.

One  $F_1$  animal was reddish brown instead of black or dun as in the other  $F_1$ . **W** was regarded, therefore, as dominant, instead of recessive as others have considered it, and ' is hypostatic to black and would therefore appear as a recessive in the first cross whenever black was involved in the cross.' The dam of this animal must have been heterozygous to black (**B**).

It was noticed from another  $F_1$  animal that **D** may affect the black gene more than the red and that **i** presumably affects the red pigment more than the black. The author believes that **i** with its effects on red pigment is present in a homozygous condition in gray animals. This condition was somewhat manifested in the back cross animals, i.e. progeny of an  $F_1$  female (Holstein-Friesian  $\times$  Brown Swiss) and Brown Swiss male cross.

One back cross female was light in colour and two somewhat darker with large amounts of red pigment ; all three had the white muzzle. Three back cross males were dark ; one had the white muzzle, but two apparently carried black and did not show the effects of **W**. One Holstein-Friesian must have been heterozygous for **Ws** as one of her cross bred daughters had the white star and one did not.

Brown Swiss and Jersey seem to have the same colour genes except for **s** which is found in some of the latter but the former lack it, the Jersey shows more red as well. Holstein-Friesians probably do not carry **Br**, brindle, as none were recovered in the  $F_1$  back crosses.

The colour genes in Brown Swiss are thought to be **bb BsBs DD** (or **BsBs ? Dd**) **ii WW inin SS brbr**. **W** is considered dominant, whereas it was formerly thought to be recessive. The dominant **Ws** is found in some Holstein-Friesians but this breed probably does not carry **Br**.  
[J. N. W.]

'Nicking' in dairy cattle. D. M. SEATH AND J. L. LUSH (1940). *J. Dairy Sci.* 23, 103.

'NICKING' is a term sometimes used to mean that some mating or group of matings were unexpectedly good ; more rarely to mean unexpectedly bad. The sire and dam may be said to have ' nicked well ' or to have ' nicked poorly ', respectively.

Chance in Mendelian segregation may, however, cause an exceptional offspring in spite of the fact that its most probable genotype will be midway between the genotypes of the two parents. Several offspring from the same parents rarely deviate from the expectation markedly in one direction as each gamete is an independent sample from the genotype of the parent and sampling errors tend to cancel each other in the average.

The possibility of the genotype of one or both parents being far better, or worse, than is supposed may give results termed ' nicking '. A genotype is known only imperfectly by the phenotype of the parent or progeny. This may be the cause of consistent good results from the mating of a particular male and female. Errors in estimating genotypes will, therefore, just as sampling errors in gene combinations in the individual mating, tend to cancel each other in the case of a large number of parents.

Deviations in the effect of a given gene caused by other genes are known as epistatic deviations, joint effects or non-additive combination effects. Certain combinations may



in this way give effects quite different from their average effects. Phylogenetically the various combinations of genes must function well enough to survive. Therefore heavy selection would occur against genes which would produce good results in certain special combinations but which in the majority of the Mendelian combinations gave bad results. Manifestations of this cause of 'nicking' are found in cases in which one sire produces good progeny with one group of dams but quite ordinary or poor progeny with another. Or if several sires are used on one group of dams their order of merit, based on the progeny, may be quite different from what it would be if they were used on another group of dams.

It is, however, not likely that sire proving would be greatly disturbed by epistatic effects since there is no effective way of getting groups of cows that are genetically uniform within groups, but contrasting from group to group. It cannot be accomplished alone by selection as the effect of one gene cannot be entirely distinguished from the effect of another gene. Joint effects involving three or more genes are even less likely to be observed with any degree of accuracy.

The authors examined the records of 13 bulls by the analysis of variance to determine whether significant differences existed among the daughters of each bull when grouped according to their maternal grandsires. No evidences of 'nicking' which would disturb the proving of any of these bulls was noted.

[J. N. W.]



## REVIEW

**Diseases of the Pig and its Husbandry.** DAVID J. ANTHONY, M.R.C.V.S., D.V.S.M. (VICT.). (London : Bailliere, Tindall and Cox, 1940. Pp. xi+272, 48 illus. 10s. 6d.).

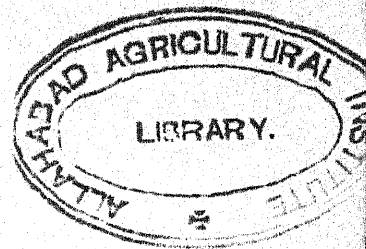
**M**R. Anthony's book fills a long-felt need for an authoritative book, in English dealing with the pig from a veterinary aspect.

Excellent descriptions are given of the various diseases of pigs and in several instances the illustrations of lesions should enable one to recognize the disease condition as it occurs in the affected animal. Eight chapters are devoted to the description of the diseases. One chapter deals with diseases scheduled in Great Britain, viz. anthrax, foot-and-mouth disease, swine fever and rabies, the next one with non-scheduled constitutional diseases which include the remaining bacterial and virus diseases and protozoan infections and another with deficiency diseases caused by the lack of minerals and vitamins. Four chapters are taken up by local diseases which include diseases of the digestive, urinogenital, respiratory, circulatory, nervous and cutaneous systems. Dental diseases also find a place amongst these diseases. The last chapter in this series is devoted to diseases caused by ecto- and endoparasites. Affections resulting from infestation with Arthropoda (flies, lice, ticks and mites), trematodes, cestodes, nematodes and acanthocephala are all described and a complete list of these parasites and the methods of examining them and their ova for identification are also given. The last chapter of the book deals with mineral and organic poisons, poisonous plants and foodstuffs, which may cause trouble in pigs, and also contains a description of anaphylactic shock as it occurs in pigs.

Though the description of diseases and their control form the bulk of the book, due prominence is also given to pig husbandry, e.g. chapters are devoted to a description of various breeds, their housing and management, breeding, feeding, dentition and ageing, signs of health, restraint and handling, market requirements, etc. This is all to the good, for it is now clearly realized that in the control of diseases of animals, their husbandry must also be the subject of study and practice.

The field covered by the publication is thus wide and comprehensive. It is attractively printed and bound and contains a vast store of practical information. Here in India, people are beginning to realize the potentialities of this industry, which yields most profitable returns, and we can warmly recommend the book to veterinarians who have to deal with pigs, to pig breeders and to others interested in this industry. [R. L. K.]





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## ORIGINAL ARTICLES

### NASAL SCHISTOSOMIASIS IN GOATS

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(With Plates IV and V)

MONTGOMERY'S [1906] pioneer work established that bilharziasis was quite common in domesticated animals in India. He showed that horse and donkey harboured *Schistosoma indicum* [Montgomery, 1906], that cattle harboured *Schistosoma bovis* [Sonsino, 1876], *Schistosoma bomfordi* [Montgomery, 1906] and *Schistosoma spindalis* [Montgomery, 1906], and that sheep harboured *Schistosoma bovis* [Sonsino, 1876] and *Schistosoma indicum* [Montgomery, 1906]. Liston and Soparkar [1918] worked out the life-history of *Schistosoma spindalis* [Montgomery, 1906] and showed that goat and buffalo also harboured this parasite. Seven years later, Fairley and Mackie [1925] studied the histo-pathology of *Schistosoma spindalis* [Montgomery, 1906] infestation in goats and established that as in bovines it affected exclusively the portal system in them and that its ova were voided only with the faeces.

The problem of schistosomiasis among animals does not appear to have attracted much attention until Malkani [1932] announced both at a public lecture and in a short note in the *Veterinary Record* that the cause of the commonly encountered nasal granuloma in cattle had been discovered to be a schistosome. This was soon followed by publications by Datta [1932], Rao [1932] and Malkani [1933]. In each of these publications it was clear that a new schistosome of cattle had been discovered, and Malkani [1933] considering it as a distinct species suggested the name *Schistosoma spindalis* var. *nasale*. Rao [1932] suggested the name *Schistosoma nasalis*. Rao [1933] and Malkani [1933] gave records of cases (though infrequent) of nasal granuloma in buffaloes due to the same parasite—*Schistosoma nasalis*.

Goats have, however, remained as yet an unsuspected host of this parasite. The object of this paper is to record the discovery that nasal schistosomiasis does occur in goats and that the causative parasite is *Schistosoma nasalis*, which is the cause of the disease in bovines and buffaloes.



On 28 March 1939, a sample of nasal discharge and small pieces of growth removed from the nostrils of a she-goat were received in the laboratory from Mr. R. R. Sarkar, Touring Veterinary Assistant Surgeon, Dinapore, with the history that the animal was showing nasal growths. Microscopical examination of direct cover slip preparations from the sample of nasal discharge revealed a large number of boomerang-shaped eggs which were indistinguishable from those found in bovine nasal schistosomiasis. Sections of the growths showed a picture similar to that seen in bovine nasal schistosomiasis but no parasites could be seen as only superficial portions of the growths had been cut. Attempts to obtain the goat for further investigation were, however, unsuccessful. The case was treated with tartar emetic and discharged as cured. On 26 June 1939 Mr. R. R. Sarkar sent to the laboratory an affected castrated he-goat (Plate IV, fig. 1), which supplied the material for the purposes of this investigation. The goat was kept under observation for a month before it was destroyed for the purpose of *post-mortem* and histo-pathological examinations.

*Features of the disease.*—It must be stated at the outset that what follows has been observed in the only case at our disposal and as it was not in an advanced stage of the disease, it is quite possible that many variations will be noticed when a large number of affected animals becomes available for observation.

The chief clinical manifestations were sneezing, coryza, congestion of and growths on the nasal mucosa and difficult respirations. The attacks of sneezing were fairly frequent. The coryza was slight and bilateral. The discharge was intermittent, small in quantity and either mucopurulent or sanguinolent. Congestion and the pimply growths were easily seen in both the nasal fossae. The growths were more or less confined to the alae nasi and the septum nasi. The growths were small and did not extend beyond an inch or so. At rest the respiratory distress was slight, on exercise it became marked. The sound emitted was not so loud as heard in bovine nasal schistosomiasis.

Nothing can yet be said about the incidence of this disease, but when more information is available it may be found that it too, like bovine nasal schistosomiasis, has a strictly regional and seasonal distribution. Of the two cases that have come under notice one was male and the other female.

*Examination of nasal discharge.*—Samples of nasal discharge were obtained on different dates from this goat. Microscopical examination of direct cover glass preparations from every sample when examined revealed among the blood corpuscles and other catarrhal products the presence of a large number of eggs (Plate V, fig. 1) all of which were of one type and like those seen in the sample sent from Dinapore were indistinguishable from those (Plate IV, fig. 3) seen in the nasal discharge from cases of bovine nasal schistosomiasis. The eggs although present in all portions of the discharge appeared to be most numerous in blood clots in which they occurred in masses; these were in all stages of development. In length they varied from  $320\mu$ - $458\mu$  and in breadth from  $48\mu$ - $64\mu$  depending upon their stage of maturity. The average length of the spine was  $12\cdot6\mu$ . The liberated miracidium did not differ from that described by Malkani [1933] in bovine nasal schistosomiasis.



FIG. 2. Microphotograph of male and female schistosomes recovered from the liver of a goat suffering from nasal granuloma. Note the tuberculated cuticle



FIG. 1. Photograph of a goat suffering from nasal granuloma

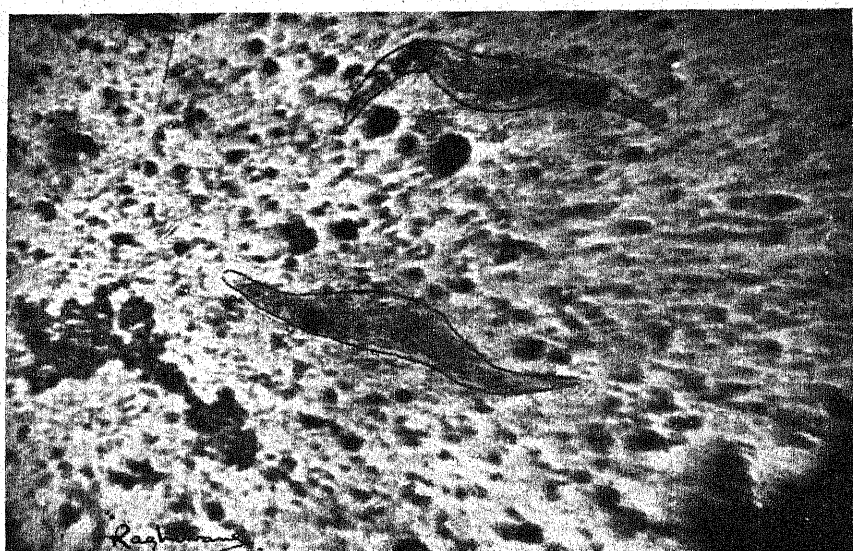


FIG. 3. Microphotograph of eggs of *Schistosoma nasalis* seen in a cover glass preparation of the nasal discharge from a case of nasal granuloma in cattle



FIG. 1. Microphotograph of characteristic eggs seen in a cover glass preparation of nasal discharge from a goat suffering from nasal granuloma



FIG. 2. Microphotograph of a section of growth showing the concentration of eosinophiles round a cavity occupied by an egg containing a well defined embryo



FIG. 3. Microphotograph of a section of a growth showing cavities containing a developed embryo in one and cut parts of embryo in others

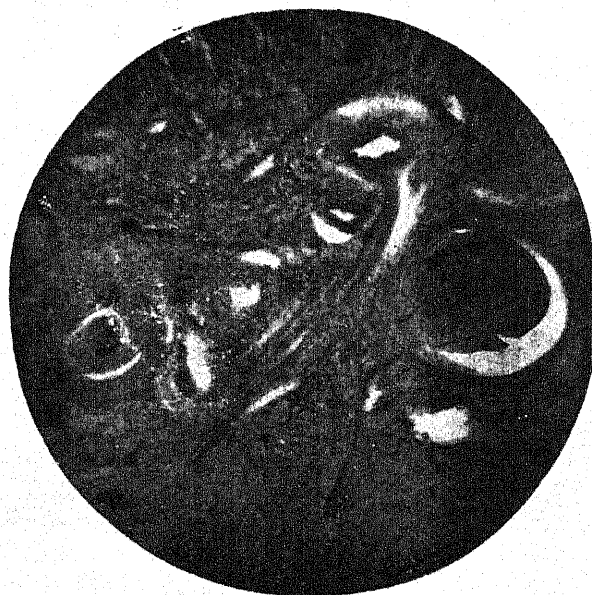


FIG. 4. Microphotograph of a section of a growth showing transverse section of the parasites (male and female in copula) *in situ*



*Examination of urine, faeces and blood.*—Samples of urine and faeces were negative for schistosome eggs. Blood smears revealed only a slight eosinophilia.

*Post-mortem examination.*—The general condition of the animal at the time of destruction was fair. On *post-mortem* examination the liver and the cortex of the kidneys were found to be slightly congested. The mouth, pharynx, oesophagus, stomach, spleen, intestines and urinary bladder showed nothing unusual. Nasal fossae showed the characteristic changes. The septum nasi and alae nasi showed the lesions, the congestion extended higher even up to the turbinate bones. The mucous membrane was oedematous and there were raised patches studded over with tiny abscesses. Small ulcers were found on the raised granulomatous growths. No growths were found in the sinuses. The growths were soft and friable and dirty grey in colour. The bases of these growths were adherent to the nasal mucosa. Their free outer surface was irregular showing ulcerations. The growths varied in size from that of a small pea to a large pea. The larynx and trachea showed nothing unusual. The lungs appeared to be normal but when incised and pressed many schistosomes were recovered. The pericardium and heart were normal.

*Collection of schistosomes.*—As Rao [1934] reports that nasal schistosomes were not recovered by him from the portal system of cattle suffering from nasal schistosomiasis, a careful examination of the liver for the parasites was, therefore, undertaken. The portal vessels were ligatured before the removal of the organ. It was washed, the knot was removed and the blood was pressed out of the vessels, to which water was added and a careful search for parasites was made. The worms were found as small white thread-like bodies coiled up in the form of letter 'C'. Only one female was found in copula (Plate IV, fig. 2). It was a hair-like structure and dark in colour.

The lungs were similarly removed from the thoracic cavity and examined. Incisions were then made into the lung tissue which was then squeezed. The water containing the blood from lungs was searched carefully for the parasites. Many single males and two females in copula were recovered.

The morphological details of the adults were as follows:—

*Male.*—Length 5-10 mm.; cuticle coarsely tuberculate; testicular glands 3-4.

*Female.*—Length 12 mm.; ovary post equatorial; length of common caecum 4.8 mm., i.e. about a third of the parasite.

In none of these females which were in copula did the uterus show any eggs.

*Morbid histology.*—As already stated small and superficial portions of the growths had been received from the Touring Veterinary Assistant Surgeon, Dinapore. Other material for histo-pathological study was obtained from the goat which was destroyed at this laboratory.

Examination of sections of these growths revealed a typical picture of highly vascular granulation tissue. Most of the sections contained only young vascular tissue showing that the growths were not of long standing. Superficially the epithelial lining was broken up at places by ulcerated areas which were covered with a meshwork of fibrin containing red blood cells,



FIG. 1. Microphotograph of characteristic eggs seen in a cover glass preparation of nasal discharge from a goat suffering from nasal granuloma



FIG. 2. Microphotograph of a section of growth showing the concentration of eosinophiles round a cavity occupied by an egg containing a well defined embryo

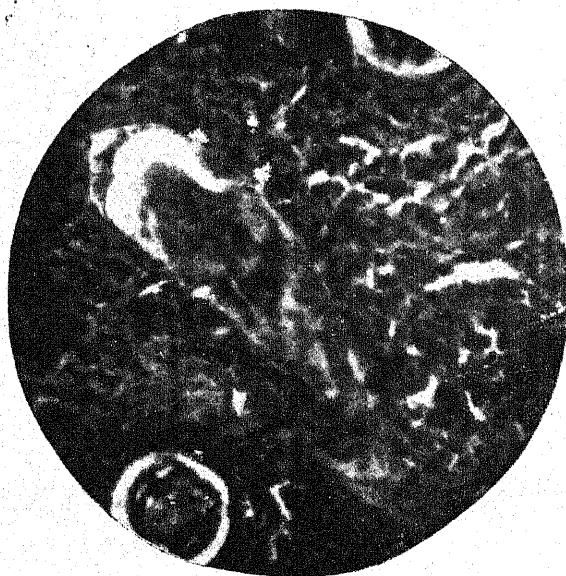


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Examination of sections of these growths revealed a typical picture of highly vascular granulation tissue. Most of the sections contained only young vascular tissue showing that the growths were not of long standing. Superficially the epithelial lining was broken up at places by ulcerated areas which were covered with a meshwork of fibrin containing red blood cells,

leucocytes and a large number of capillary loops. Deeper layers showed marked cellular infiltration with round, epithelial, a few polymorphonuclear but mostly eosinophile cells. The eosinophile infiltration was noticeable around cavities. Many of these cavities were empty and some of them had a striking resemblance to the shape of the eggs of the parasite. Others were seen to be occupied by eggs enclosing well-defined embryos (Plate V, fig. 2) or even fully developed miracidium (Plate V, fig. 3). The large blood vessels situated deeply in the affected tissue, specially the veins, showed transverse sections of schistosomes, some of them single, others in copula (Plate V, fig. 4). Such veins showed considerable dilatation and their endothelium was also thickened. Around some of these veins one could see the same kind of cellular infiltration with formation of large plasmoidal giant cells, eosinophile infiltration and fibrosis as seen round the eggs. In some parts the arteries showed varying degrees of endarteritis.

The morbid histology was thus similar to the one seen by Malkani [1933] in cases of bovine nasal schistosomiasis.

*Discussion.*—The results of this investigation have shown that nasal schistosomiasis occurs in goats. Further, the schistosome responsible for this disease in goats is identical with *Schistosoma nasalis* [Rao, 1932]; this is proved by a comparison of the photographs and morphological details of the adult parasite and its eggs obtained from goat with those from cattle affected with nasal schistosomiasis.

There appears to be some difference of opinion as regards the positions in which nasal schistosomes are found in their host. Malkani [1933] found in bovines most of the schistosomes, both single and in copula, from the liver and the examination of the *in-utero* eggs showed that they were exactly like those seen in the nasal discharge. Rao [1934] reports that he has not been able to find the nasal schistosomes in the portal or mesenteric vessels of animals examined in abattoirs or in calves experimentally infested with nasal schistosomes and goes on to state 'these parasites do not develop in the portal or mesenteric vessels and that they choose the nasal veins only as their habitat and develop there'. The author has obtained many schistosomes from the liver as well as the lungs of the goat under observation; two from the former and one from the latter situation were in copula. A comparison of schistosomes from both organs showed that they were identical and had the same tuberculated cuticle. This evidence together with the absence of any eggs of *Schistosoma spindalis* [Montgomery, 1906] in faeces would point to these parasites obtained from the liver and the lungs being probably *Schistosoma nasalis* but definite opinion as to their identity must be held in abeyance till parasites containing eggs are obtained for study.

#### CONCLUSIONS

1. Nasal schistosomiasis occurs in goats.
2. The species of schistosome responsible for this caprine nasal schistosomiasis is *Schistosoma nasalis* which is also responsible for nasal schistosomiasis in cattle.

3. The goat like the buffalo must therefore be taken into consideration in the eradication campaign against the more common bovine nasal schistosomiasis.

## ACKNOWLEDGMENTS

Thanks are due to Mr. M. I. Malik, B.Sc., M.R.C.V.S., Principal of the Bihar Veterinary College, for the facilities afforded for this investigation. We wish to place on record our appreciation of the help received from Messrs. K. S. Sankaram (Demonstrator), Raghuvans Bhushan (Artist) and R. R. Sarkar, Touring Veterinary Assistant Surgeon, Dinapore.

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# CONCENTRATION OF VITAMIN C (ASCORBIC ACID) IN THE TISSUES OF FARM ANIMALS IN HEALTH AND DISEASE\*

BY

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(With one text-figure)

CONSIDERABLE interest in the rôle played by vitamin C in the animal system has been caused by the recent discovery that the vitamin C requirement of the body is very greatly increased in several infectious diseases. Heise and Martin [1936] found that patients suffering from tuberculosis excrete in the urine less vitamin C than is excreted by healthy persons on a similar diet, and the vitamin C requirement of such patients is much greater than the normal. Similar findings were recorded by other workers in the case of a variety of infectious diseases such as pneumonia [Harde, Rothstein and Ratish, 1935] and rheumatoid arthritis [Abbassy and Harris, 1937].

As very little is known about the rôle played by vitamin C in the animal body, the cause of the increased requirement in these diseases cannot be explained satisfactorily. The most plausible theory is that this vitamin may have some anti-infective action within the body and probably reacts with the toxins produced by micro-organisms. Some support to this theory has been given by Heise and Schwartz [1937], who observed a small improvement in tubercular patients who were given vitamin C. King and Menten [1935] and Greenwald and Harde [1935] also found a similar beneficial effect from the administration of vitamin C in diphtheria while Jungeblut [1939] obtained similar results in poliomyelitis. Other workers [Sendroy and Schultz, 1936; Parsons, 1938; Baumann and Rappolt, 1937; Zilva, 1937] have, however, failed to observe any such improvement in any disease through the administration of vitamin C, and they ascribe the heightened requirement of the body to the increased catabolic destruction of the vitamin owing to pyrexia.

So far, however, no work seems to have been done to find out the diminution, if any, in the vitamin C concentration of the various tissues of common domestic animals suffering from infectious diseases. The point is of great interest since animals such as cows, horses, goats and fowls are known to live for indefinite periods in perfect health without the inclusion of any vitamin C in their diet, and are supposed to be able to synthesize this vitamin within their bodies from unknown ingredients in their feed. In fact, the only species which require vitamin C in their diet are men, monkeys and guinea-pigs.

\*This work was carried out in the Pathology and Bacteriology section of the Imperial Veterinary Research Institute, Mukteswar.

The present work was taken in hand in order to find out whether the endogenous production of vitamin C in livestock is sufficient to meet the enhanced requirement of the system during disease. A number of interesting facts were revealed and it was found that in many diseased conditions the vitamin C concentration in the blood and other tissues was much lower than the corresponding values in normal animals, proving thereby that the capacity of the animals to synthesize this vitamin is distinctly restricted.

## EXPERIMENTAL

The vitamin C (ascorbic acid) contents of the tissues were estimated according to the method of Birch, Harris and Ray [1933]. The capsules of the organs were removed and the central portions dried between pieces of filter paper. Weighed amounts of tissue were then thoroughly ground up with 20 per cent trichloroacetic acid and sand and the mixture made up to a definite volume so that the final suspension contained 5 per cent trichloroacetic acid. The filtrate was titrated against a definite volume of an aqueous solution of 2:6 dichlorophenol indophenol, standardised against pure ascorbic acid. In the case of blood plasma, 7 c.c. of plasma were added to 3 c.c. of 20 per cent trichloroacetic acid, mixed thoroughly and centrifuged rapidly for fifteen minutes. Four c.c. of the clear supernatant was titrated against a dilute standard solution of 2:6 dichlorophenol indophenol. A blank was run at the same time with diluted trichloroacetic acid and the amount of dye required to obtain a pink colour was subtracted from the amount required by the plasma sample. Except where otherwise mentioned, all blood samples were collected from animals showing definite symptoms of the particular diseases under study, and the liver and spleen samples were taken as soon as possible after the death of the animals. Any sample showing the slightest sign of putrefaction was rejected. Both the normal and diseased animals were kept as far as practicable on the same type of ration.

The results obtained were as follows:

TABLE I

*Concentration of ascorbic acid in the various tissues of normal and diseased animals*

Nature of disease	Concentration of ascorbic acid in tissues examined		
	Blood plasma mg/100 c.c.	Liver mg/gram	Spleen mg/gram
<i>Hill-bulls</i>			
Normal	0.34—0.77 (0.50)	0.30—0.45 (0.39)	0.22—0.35 (0.26)



TABLE I—*contd.*

Nature of disease	Concentration of ascorbic acid in tissues examined		
	Blood plasma mg/100 c.c.	Liver mg/gram	Spleen mg/gram
<i>Hill-bulls</i>			
Rinderpest	0.06—0.39 (0.19)	0.15—0.21 (0.18)	0.06—0.10 (0.08)
Acute theileriasis	0.25—0.51 (0.43)	0.16—0.28 (0.23)	0.11—0.27 (0.18)
Blackquarter	..	0.04—0.09 (0.07)	0.03—0.05 (0.04)
<i>Goats</i>			
Normal	0.40—1.14 (0.74)	0.28—0.51 (0.40)	0.14—0.31 (0.28)
Rinderpest	0.10—0.74 (0.48)	0.14—0.39 (0.25)	0.08—0.21 (0.13)
<i>Ponies</i>			
Normal	0.45—0.82 (0.61)	..	..
Helminthic infestation	0.00—0.28 (0.17)	..	..
<i>Fowls</i>			
Normal	..	0.28—0.45 (0.41)	0.30—0.38 (0.31)
Ranikhet disease	..	0.38—0.56 (0.47)	0.27—0.44 (0.36)

NOTE.—The figures within brackets represent average values.

The values for the concentration of the vitamin in the tissues of normal animals were found to agree reasonably well with the figures given by workers in the western hemisphere [Birch, Harris and Ray, 1933 ; Bessey and King, 1933 ; Svirebely, 1933].

It will also be seen that in hill-bulls and horses, certain bacterial or virus infections or helminthic infestations appear to lower vitamin C in the blood and to deplete considerably the store of this vitamin in the liver and spleen. It is probable, therefore, that the rate of destruction or utilization of the vitamin under pathological conditions is much greater than the rate of synthesis of vitamin C by these animals. On the other hand, protozoal infections such as theileriasis appear to have relatively much less effect on the vitamin C concentration of either the blood or the spleen. This difference between the effect of a typical virus disease such as rinderpest and that of acute theileriasis on the concentration of vitamin C in the blood is clear from Table II.

TABLE II

*Statistical treatment of figures in Table I*

Groups	<i>t</i>	Degrees of freedom	Remarks
A	55.28	28	Very significant
B	13.57	22	Significant

Group A. Comparison between the concentrations of vitamin C in normal animals and in animals suffering from rinderpest.

Group B. Similar comparison in normal animals and animals suffering from acute theileriasis.

The results show that the degree of depletion in rinderpest is more than four times as great as in acute theileriasis.

In fowls, the concentration of vitamin C in the liver and spleen did not change when the birds had even died from such a virulent disease as Ranikhet disease. If anything, the concentration was found to have increased beyond that found in normal birds. The reason for this sharp difference between fowls and other animals is not clear.

In the case of hill-bulls suffering from rinderpest it was also found (Table III) that the fall in the concentration of vitamin C in the blood was roughly proportional to the degree of severity of the infection. In this connection, it may be pointed out that in goats, which suffer from an attenuated form of rinderpest, the drop in the concentration of vitamin C in blood is much smaller than that in bulls.

TABLE III

*Correlation between vitamin C concentration of blood and severity of rinderpest infection*

Number of hill-bull	Concentration of vitamin C (mg. per 100 c.c. blood plasma)	Degree of infection
43	0.064	+++++ (Very severe)
44	0.220	+++++ (Do.)
126	0.048	+++++ (Do.)
132	0.220	+++++ (Do.)
87	0.310	++ (Mild)
94	0.240	++++ (Severe)
148	0.39	0 (Nil)
66	0.285	+
32	0.125	+++++ (Very severe)

In order to find out whether the fall in the ascorbic acid concentration of blood is due to the high temperature run by the animals, a day-to-day estimation was made of the blood of normal animals, of animals inoculated with rinderpest virus and of animals infected with acute theileriasis. The results are depicted graphically in Fig. 1. Each curve represents the average of the figures comprising each group. It will be seen that the concentration of vitamin C in the blood of animals inoculated with rinderpest began to fall on the second day, when the temperature of the animals was still normal. Moreover, in cases of theileriasis the concentration did not fall below the initial levels even though the animals were running high temperature. It may therefore, be inferred that the reduction in vitamin C in rinderpest is mainly due to the action of virus and is not associated with pyrexia. Further, the curves definitely prove that acute theileriasis has little effect on the vitamin C concentration of the blood of infected animals.

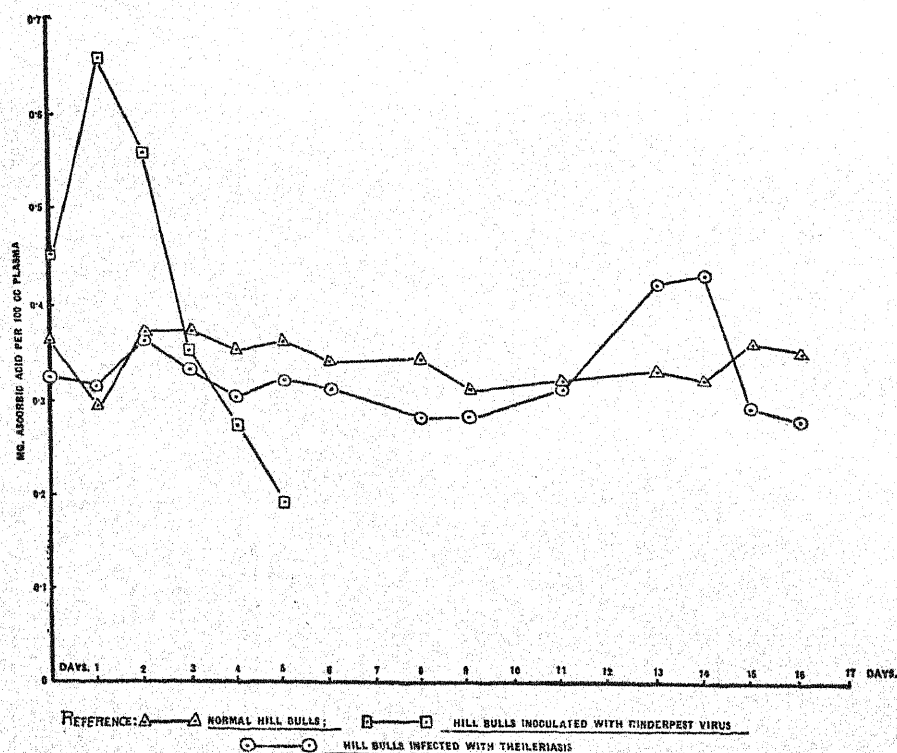


Fig. 1. Rate of variation in the vitamin C concentration of blood plasma in normal and infected animals.

A curious feature of the curves is the sharp rise in vitamin C within 24 hours of inoculating rinderpest virus. This rise was observed in all the animals examined. A similar but smaller rise is also seen in the curve for cases of theileriasis after the thirteenth day, that is two or three days before the protozoa appear in the blood stream. This rise in the concentration of the vitamin appears to be a mobilising action on the part of the animal system in order to strengthen the defensive mechanism against the invading organisms.

#### SUMMARY

A decrease in the concentration of vitamin C (ascorbic acid) in the blood, liver and spleen of animals suffering or dying from a number of infectious diseases such as rinderpest, blackquarter and helminthiasis has been observed. In certain other diseases, such as acute theileriasis in bulls or Ranikhet disease in fowls, little or no such reduction was seen.

In rinderpest, the diminution in the vitamin C concentration of the blood was found to run parallel with the degree of severity of the infection though it was not associated with rise of temperature.

A sharp rise in the concentration of the vitamin in the blood was observed within 24 hours of inoculating rinderpest virus. A similar but smaller rise was also seen after the thirteenth day in animals infected with acute theileriasis. The significance of these findings is discussed.

#### ACKNOWLEDGEMENTS

My thanks are due to Mr. J. R. Haddow, Officer-in-charge of the Pathology and Bacteriology Section of this Institute, for his constant advice and guidance, to Dr. H. N. Ray, Systematic Protozoologist and to Mr. M. K. Sreenivasan, Veterinary Deputy Superintendent, for kindly supplying me with blood from animals suffering from acute theileriasis and rinderpest.

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# EXPERIMENTS ON THE TRANSMISSION OF RINDERPEST THROUGH THE AGENCY OF *TABANUS ORIENTIS*, WITH REMARKS ON THE FEEDING HABITS OF THIS FLY

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(With 2 text-figures)

## INTRODUCTION

RINDERPEST, being one of the oldest diseases recognized in this country as causing enormous losses among cattle every year, it would be imagined that the mode of its transmission has been fully investigated by now, but a perusal of the available literature on the subject shows that this is not so. Arthropods as transmitting agents of this disease have been mentioned by various observers, but very little appears to have been done to find out whether they actually transmit the disease and set up an outbreak. The only efforts made in this direction were by Sen [1925, 1937], Hornby [1926] and Bhatia [1935]. After the reader has gone through the account of the experiments carried out by the above workers and of those detailed in this article, he would not only hesitate to form any definite opinion but also find certain conclusions as being incompatible.

A few more workers have conducted experiments which aimed only at determining the duration of the viability of the rinderpest virus in the body of a tick or a fly or in the urine, faeces, etc. In most cases emulsion of the fly or the tick, after it had fed on the infected animal or on its blood or faeces, was inoculated into healthy cattle to see if the disease could be reproduced in this manner, but in no case was the disease transmitted through a bite. Thus Ward and his collaborators [1914] experimented on the viability of the virus in urine and faeces of the infected animals under various conditions, whilst Shilston [1917] carried out similar experiments with meat, bone marrow and blood. Curasson [1922] succeeded in reproducing the disease by inoculating the emulsion of an infected engorged *Ixodes ricinus* tick immediately on removal from its host, but the result was negative when this was done an hour after removal. His work with *Tabanus* will be mentioned later in this article. DeSouza [1924] found that the tick, *Boophilus annulatus*, fed on an infected animal was virulent on injection after seven days.

Work on the actual transmission of rinderpest through bites of insects was first undertaken by Sen [1925]. With *Aedes (Stegomyia) albopicta* the results of his experiments on hill bulls were negative. With *Musca domestica* he obtained negative results when flies fed on infective material (such as nasal discharge, blood and faeces of infected animals) were allowed to come in contact with such parts of the body as seemed likely to serve as avenues of infection under natural conditions, but the results were positive when such infective flies were inoculated into susceptible bulls. In his third series of experiments he employed *Linognathus vituli*. Here, too, the results were negative



when infected lice were transplanted on a healthy bull but positive when an emulsion of these was inoculated. Hornby [1926] succeeded only in a single instance in transmitting rinderpest in East Africa through the bites of *Glossina morsitans*. Crawford [1933], in Ceylon, suggested that in the absence of *Glossina* the larger biting flies might transmit the disease in India. Working on this suggestion, Bhatia [1935] was the first to carry out experiments with *Tabanus orientis* and *Stomoxys calcitrans*, using the interrupted method of feeding. He conducted four experiments with *T. orientis*, feeding 6 flies in the first, 8 in the second, 18 in the third with negative results and 36 in the fourth where he claims to have succeeded in transmitting the disease. With *S. calcitrans*, however, his findings were negative throughout. Sen and Abdus Salam [1937] also report negative results with *S. calcitrans*. They made 411 experimentally engorged flies (fed singly) to bite a healthy animal over a period of 23 days and 670 flies (fed *en masse*) in 6 days, thus bringing the total to 1081 over a period of 29 days.

The experiments described in this article were carried out with the object of obtaining an indication as to the extent to which transmission through biting flies is possible under field conditions, the only species of fly used in these experiments being *T. orientis*. The spread of a fly-borne disease depends largely on the feeding habit of the vector and, since very little is known about this habit in *T. orientis*, it was considered desirable also to deal with this aspect of the problem.

#### MATERIAL AND METHOD

The flies were mostly secured from the pastures frequented by healthy buffaloes every morning. They were kept in ordinary mosquito netting cages and starved for 24-48 hours. A thick layer of absorbent cotton soaked with water was kept on the roof of each cage and this in turn was covered over with a damp cloth, in order to provide the flies with a constant supply of moisture. During the day these cages were partly exposed to sunlight and a few dry twigs were also placed in them in order to simulate the natural conditions as far as possible.

After the lapse of the above-mentioned period their wings were clipped half and they were then fed on a bull artificially infected with rinderpest. The host, if quiet, was made to stand; otherwise it was cast and properly secured. An area about 6-in.  $\times$  6-in. on the back in the former and on the abdomen in the latter case was shaved and wiped clean. The feeding of flies was always conducted in shade. Six to ten flies at a time were left on the prepared site and covered over with a glass beaker, a watch being kept to study their behaviour.

An animal at the height of temperature reaction was selected for providing the infective feed and used for four consecutive days for feeding the flies and it was then replaced by another that had been injected with virus four days previously. The flies that started feeding were allowed about one-and-a-half to two minutes for the feed and then interrupted. When a fly had actually fed, a large drop of blood appeared at the site of bite and it was then allowed to complete its feed on a healthy bull which was kept close at hand. If, however,

no blood appeared the fly was allowed again to continue its feed for another minute or so until blood appeared. Every individual feeder was treated in this manner. The interval between the infective and healthy feeds was restricted to just a few seconds. The experimental bulls, except when brought for feeding flies, were kept in *chappars* a good distance away from the rinderpest shed.

#### RINDERPEST TRANSMISSION EXPERIMENTS WITH *T. ORIENTIS*

The results of two transmission experiments are summarized in Tables I and II.

TABLE I

##### First experiment

Date and number of flies collected	Number of rinderpest infested bull used and number of flies partially fed	Number of flies induced to complete their feed on healthy bull No. 176	Date of feeding	Remarks	Result
(1939)			(1939)		
12th May: 50 flies . . .	H. B. 511: 27 flies	27	13th May	Flies were starved for 24-48 hours before feed. The interval between the infective and healthy feeds was a few seconds.	Negative
13th May: 56 flies . . .	" 34 "	34	14th May		
14th May: 50 flies . . .	" 30 "	30	15th May		
15th May: 48 flies . . .	" 31 "	31	16th May		
16th May: 40 flies . . .	H. B. 596: 26 flies	26	17th May		
17th May: 25 flies . . .	" 15 "	15	18th May		
18th May: 60 flies . . .	" 40 "	40	19th May		
19th May: 52 flies . . .	" 32 "	32	20th May		
20th May: 41 flies . . .	H. B. 271: 22 flies	22	21st May		
21st May: 30 flies . . .	" 21 "	21	22nd May		
22nd May: 39 flies . . .	" 25 "	25	23rd May		
23rd May: 18 flies . . .	30 flies H. B. 470: 22 flies	22	25th May		
24th May: 14 flies . . .		19	26th May		
25th May: 25 flies . . .		19	27th May		
26th May: 31 flies . . .		19			
27th May: 35 flies . . .	65 flies H. B. 31: 50 flies	50	29th May		
28th May: 30 flies . . .		40	30th May		
30th May: 42 flies . . .		33	31st May		
31st May: 37 flies . . .		27	1st June		
1st June: 50 flies . . .	H. B. 552: 38 flies	38	2nd June		
2nd June: 40 flies . . .	" 27 "	27	3rd June		
3rd June: 11 flies . . .	41 flies " 34 "	34	5th June		
4th June: 30 flies . . .					
5th June: 45 flies . . .	H. B. 437: 36 flies	36	6th June		
6th June: 60 flies . . .	" 40 "	40	7th June		
7th June: 75 flies . . .	" 45 "	45	8th June		
8th June: 35 flies . . .	" 24 "	24	9th June		
9th June: 39 flies . . .	H. B. 399: 24 flies	24	10th June		
10th June: 20 flies . . .	44 flies " 40 "	40	12th June		
11th June: 24 flies . . .					

It will be seen from Table I that a total of 1210 wild flies were used in the experiment and that 821 of these were partially fed on rinderpest infected animals and were allowed to complete their feed on a healthy bull which was thus subjected to the bites of infected flies over a period of 27 days, but the result was negative. The bull was subsequently tested with rinderpest virus and was found susceptible to the disease (Fig. 1).

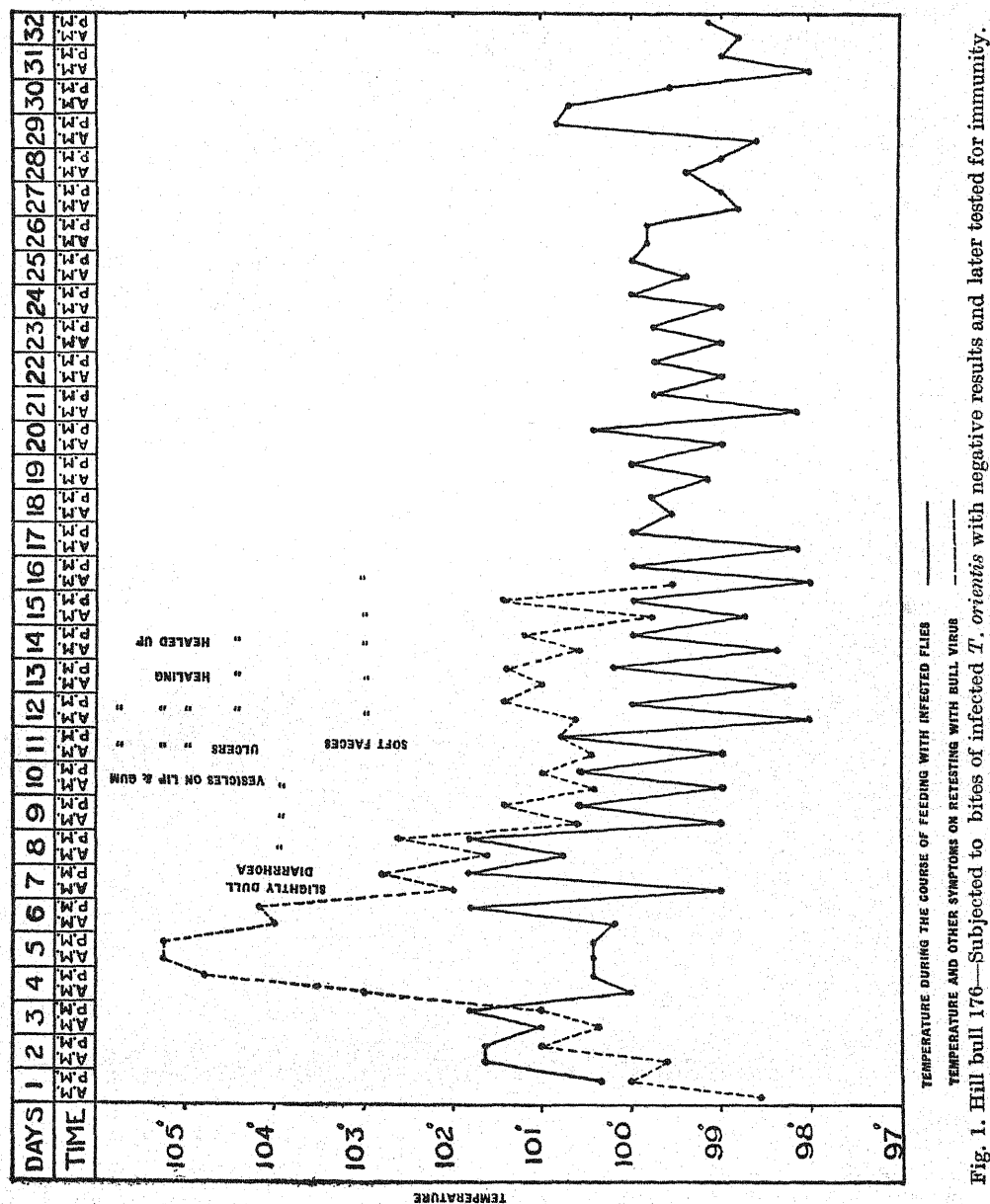


TABLE II

## Second experiment

Date and number of flies collected	Number of rinderpest infected, bull used and number of flies partially fed	Number of flies induced to complete their feed on healthy bull No. 25	Date of feeding	Remarks	Result
(1930)			(1930)		
14th June : 25 flies	H. B. No. 561 : 19 flies	19	15th June	Flies were starved for 24-48 hours before feed. The interval between the infective and healthy feeds was a few seconds	Positive
15th June : 20 flies	H. B. No. 561 : 11 flies	11	17th June		
16th June : 4 flies	H. B. No. 11 : 24 flies	24	19th June		

From Table II it will be observed that a total of 54 infected flies were fed on a healthy bull on three alternate days with positive results. The animal developed a mild thermal reaction followed by mouth lesions and diarrhoea resulting in death (Fig. 2). The *post-mortem* examination revealed typical lesions of rinderpest.

During transmission experiments the healthy and the infected bulls were stationed about three yards apart from each other in order that the interval between the infective and healthy feeds of the flies might be reduced to the minimum. It might be a matter of speculation as to whether the positive result obtained in the case of hill bull No. 25 might not be attributed to the bull contracting the infection through contact rather than through infective bites. Experimental evidence [Cooper, 1932] available on this point shows, however, that it is unlikely that rinderpest can be transmitted in this manner, except when very close contact is maintained for a period of not less than three days. As remarked by Cooper, it is possible to transmit this disease only if the closest possible contact was established and even then it would fail to develop in as many as half the number of animals. By 'closest possible' contact the author meant common feeding, watering and housing.

OBSERVATIONS ON THE FEEDING HABITS OF *T. ORIENTIS*

A series of experiments was carried out to determine the relative frequency with which *T. orientis* would feed by the interrupted and uninterrupted methods. The first experiment was commenced with eight flies, out of which only one was noticed to feed. It took about 5 minutes to complete its feed. It was separated from the rest which latter were starved for another 24 hours and during this period two of them died. The remaining five were again tried for half an hour on the following day, but as none of them was observed feeding, they were starved for another 24 hours and during this period two more were found dead. The remaining three were afforded another chance to feed on the third day morning but without success. On the fourth day,

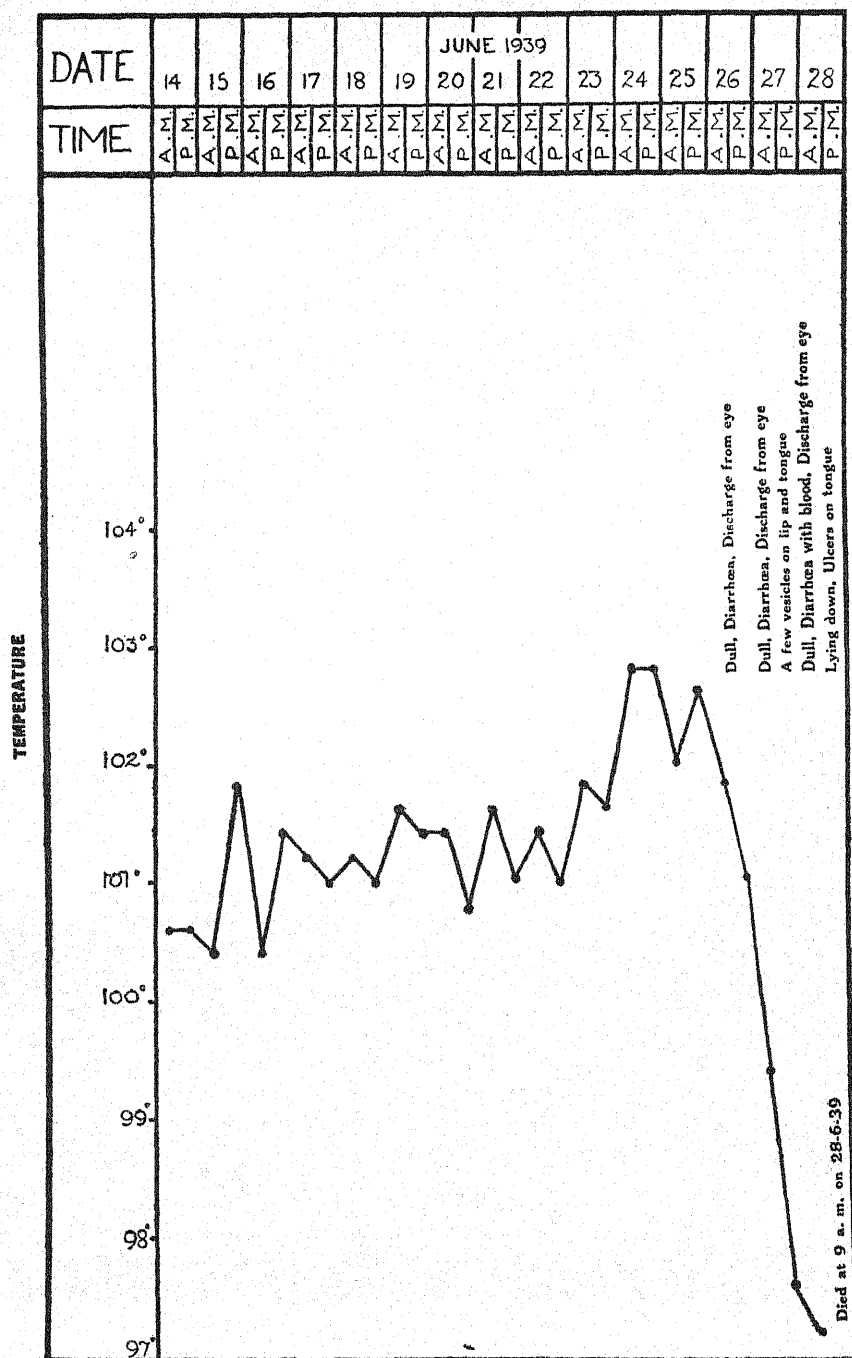


Fig. 2. Hill bull 25—Subjected to bites of infected *T. orientis* with positive results.



only one was left which refused to feed and died the following night, indicating thereby that those flies which refused to feed on the first day had possibly a meal of blood before they were captured. The one that fed on the first day was again tried on the fourth day for about an hour when it showed no inclination to feed and was found dead the next morning.

A second experiment with a further batch of 60 flies was carried out and 40 out of them were seen to feed, out of which 35 were probably newly-emerged, as judged by the colouration of their eyes. The duration of feed in these cases ranged from 4 to 10 minutes. Thirty out of these 40 (the rest being dead) were again tried on the fourth day when only one of them was noticed to feed for about a minute and a half. These 30, along with the remainder of the first day's unfed lot, were afterwards tried every day for the next 9 days till all of them died. During this period not one of them was seen to feed.

The foregoing experiments were repeated during May and June of the following year and in these a total of nearly 80 flies were used. Of this number, only 6 could be induced to take two complete feeds, the interval between these feeds being about 72 hours in each case.

In another experiment the interrupted method of feeding was employed, using a batch of 17 flies. Eleven started feeding and were allowed to continue the feed for about half a minute. These were then separated and the rest discarded. Next morning 2 were dull and refused to feed, while the other nine commenced feeding again but were interrupted after about a minute. The dull ones died during the night. On the third day, 7 out of the 9 were living and 6 of these were observed to feed once again and were allowed to do so for another half a minute, while the seventh was injured and subsequently died. On the fourth day, 4 out of 6 fed readily and were interrupted after a minute, while the other two refused to feed and died, one in the afternoon and the other the following morning. On the fifth day, only 3 survived. Two of these started feeding and were disturbed after half a minute, while the third was allowed to complete its feed and took 3 minutes to do so. This fly was kept separately. On the sixth day, the incompletely fed ones again commenced feeding but were interrupted after half a minute. On the other hand, the completely fed one refused to feed again although allowed half an hour to do so, once in the morning and again in the afternoon. On the seventh day, only one of the interrupted feeders and the one that had been completely engorged were found living. The former could be induced to feed again only for about a minute and never fed again during the following five days of its life in spite of repeated trials, while the latter refused to feed altogether and was found dead the next morning.

From the observations recorded above, it would appear that, while the fly feeds readily a number of times by the interrupted method, it rarely takes more than one complete feed in captivity. This would seem somewhat remarkable in view of what is known concerning the feeding habits of other species of *Tabanus*. Thus, according to Patton and Cragg [1913], *Tabanids* feed every third day, while Cross and Patel [1921] observed that *Tabanus nemocallosus* sucked blood four times within 5 days and ten times within 16 days.

During the course of the above experiments it was observed that it took hardly 5 minutes to induce the flies to feed on a black bull, whereas it usually took 10-20 minutes to make them feed on a brown bull. This finding was further supported by the fact that these flies were always caught in areas where buffaloes were grazed, but were less common in places frequented by other cattle. In view of the above observation, black bulls were, as far as possible, selected for the subsequent experiments.

Incidentally a few observations were made on the behaviour of *T. orientis* under natural conditions, towards animals grazing out in the field. In one instance a fly attempted to attach itself to the hind limb of a bull but was prevented from doing so by the lashing of its tail. The tail of the animal was then secured by an attendant, while the fly was still hovering over it and it now settled on the hind shin and thrust its proboscis into the skin. At its initial stage, the bite caused some discomfort to the animal, as was evidenced by the fact that it shook the limb in an ineffectual attempt to dislodge the fly but later it ceased to do so, even after the tail was let loose, and the fly completed its feed in about 5 minutes. In order to find out whether this particular fly would feed again it was caught and repeated attempts at feeding it proved futile.

In another case a fly was seen alighting on the inside of the hock joint of a bul. In spite of the lashing of the tail and the kicking of the legs by the animal the fly succeeded in fixing itself. Immediately after, the animal became quiet and continued grazing and the fly, after completing its feed, flew off, leaving a drop of blood at the site of puncture. It was, however, captured and all efforts to feed it on subsequent days proved unsuccessful.

About thirty other observations were made on the feeding habits of *T. orientis* under field conditions at Mukteswar and they were essentially of the nature described above. It would therefore seem improbable that *T. orientis* plays any important rôle in the spread of rinderpest under field conditions in India, and inferentially this remark applies to other species of the family of Tabanidae. It is of interest that Patton and Cragg [1913] would appear, in essence, to share the views expressed above, for these workers observe as follows: 'Most biting flies take a full meal at each feed, and seldom feed more than once a day, so that the parasite cannot be passed on to a second host unless it is capable of living during this period in what would appear, judging from the behaviour of such organisms under laboratory conditions, to be a very unfavourable environment; or if it happened that the fly was interrupted in its meal, and settled on another individual to complete it. The intermittent habit of feeding of the non-biting muscids suggests that they are more likely to spread infection in this way than are true biting flies'.

#### VIABILITY OF RINDERPEST VIRUS IN *T. ORIENTIS*

A series of experiments was conducted to determine the viability of rinderpest virus in the body of *T. orientis* after artificial feeding on infected bulls.

Curasson [1922] was the earliest to undertake such viability tests in Tabanids (species not stated). In a single instance he was able to transmit the disease by inoculation of an emulsion of proboscides removed from flies immediately after their infective feed on an animal on the third day of fever. The result of such inoculation, however, proved negative when the emulsion was made and injected 15 minutes after the infective feed. Negative results were also obtained when there was an interval of three quarters of an hour.

In the present experiments a lot of 10 dead flies, fed on an infected animal at the height of thermal reaction, was mashed 48 hours after their feed in 10 c.c. of normal saline solution. The emulsion was strained, filtered and inoculated subcutaneously into a healthy bull without producing any effect.

The inoculation was then repeated using 10 living flies but the results were likewise negative.

A third lot of 10 flies was emulsified 20 hours after the feed and on inoculation produced typical symptoms of rinderpest after an incubation period of 5 days. The animal, however, recovered and was subsequently subjected to immunity test and proved to be immune.

Finally, the experiment was repeated with an interval of 30 hours and this likewise yielded positive results.

The above observations are summarized in Table III.

TABLE III

Rinderpest infected hill bull No.	Date and number of flies fed	Date of inoculation	Interval	Results	Incubation period	Remarks
	(1939)	(1939)	(Hrs.)		(Days)	
H. B. 176	13th May : 10 flies	15th May	48	Negative	...	All flies dead
H. B. 31	30th May : 10 flies	1st June	48	Negative	...	All flies living
H. B. 596	19th May : 10 flies	20th May	20	Positive	5	9 flies living and one dead
H. B. 561	15th June : 10 flies	16th June	30	Positive	4	All flies living

It was intended to determine the longest period for which the virus would remain viable but due to rains having set in, no flies could be secured and further experimentation could not therefore be undertaken.

#### SUMMARY AND CONCLUSIONS

1. A total of 821 wild *T. orientis* flies were partially fed on rinderpest infected animals and allowed to complete their feed on a healthy bull, but the latter did not develop the infection. When subsequently tested for immunity by the inoculation of rinderpest virus, the bull proved susceptible to the disease.

Another healthy bull was subjected to only 54 infective bites and this animal developed typical symptoms of the disease, which eventually progressed to a fatal issue.

2. Observations on the feeding habits of *T. orientis* have shown that the fly seldom takes more than one complete feed in captivity and that, under field conditions, it usually enjoys an uninterrupted feed until it is fully engorged. These facts make it impossible for this fly to play any important rôle in the spread of rinderpest.

3. The virus of rinderpest is viable in the body of the fly for a minimum period of 30 hours and inert at 48 hours.

#### ACKNOWLEDGEMENTS

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# CAPILLARIA BOVIS SCHNYDER, 1906 FROM THE INTESTINE OF A CALF AT MADRAS

BY

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(With six text-figures)

ZEDER [1800] established the genus *Capillaria* on material from poultry, though Schrank [1790] was the earlier to record the first member of the genus with *Capillaria (Trichocephalus) anatus* from *Anser ferus*, as the type species. Railliet [1915] accepted it as the type genus of a new sub-family Capillarinae, under the family Trichuridae. Representatives of this genus are parasitic in the intestine, liver or urinary bladder of birds, mammals, etc.

Only four species of *Capillaria* have been known to be parasitic in the domesticated ruminants. Ransom [1911] described two of these, *C. longipes* and *C. brevipes* from the small intestine of sheep in the United States. Snyder [1906] recorded the species *C. bovis* from the ox in Switzerland, giving a brief description of the worms. Morgan [1925] obtained one female of this genus from the ox in Wales, which he found allied to *C. bovis* and *C. longipes*, thus failing to arrive at a conclusive identity. Bhalerao [1933] described *C. bilobata* from the abomasum of hill bulls at Mukteswar.

The present collection of one male and two females was made from the small intestine of an experimental calf, at the Madras Veterinary College; in their main features, the specimens obtained resemble those of *C. bovis* and this opportunity is taken to record it for the first time in this presidency and possibly in India.

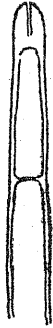
The worm has a capillary body; the anterior shorter portion contains the oesophagus; the posterior, very little longer, is only slightly thicker than the anterior. The head end (Fig. 1) is smooth and rounded; mouth is simple. Oesophagus is long, gradually increasing in size posteriorly. It consists (Fig. 2) of a delicate tube apparently running through the centre of a chain of cells. The cells in the anterior portion are not clearly made out; the posterior cells, which are first small, gradually increase in size towards the end of the oesophagus. At the junction of the oesophagus and the intestine (Fig. 3) are situated the yellowish-brown pyriform glands. The bacillary bands on the cuticle are very indistinct in the preserved specimens. The intestine takes an almost straight course and passes into a muscular rectum opening by the anus which is sub-terminal (Figs. 4 and 6).



**CAPILLARIA BOVIS (FEMALE)**

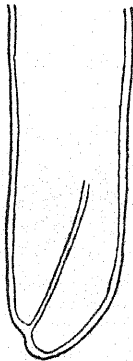
**Fig. 2**

**Fig. 1**



**HEAD END**

**Fig. 4**



**TAIL END**

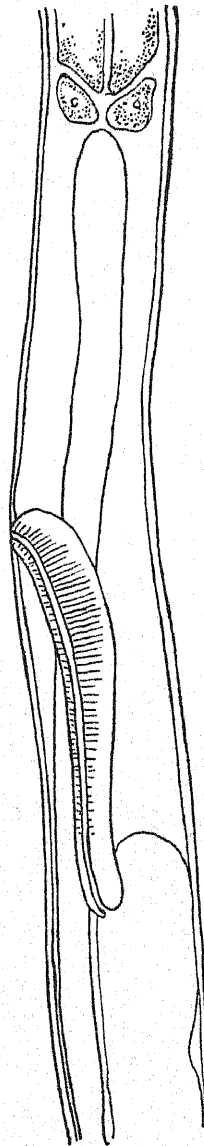
**Fig. 5**



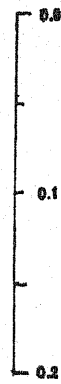
**EGGS**

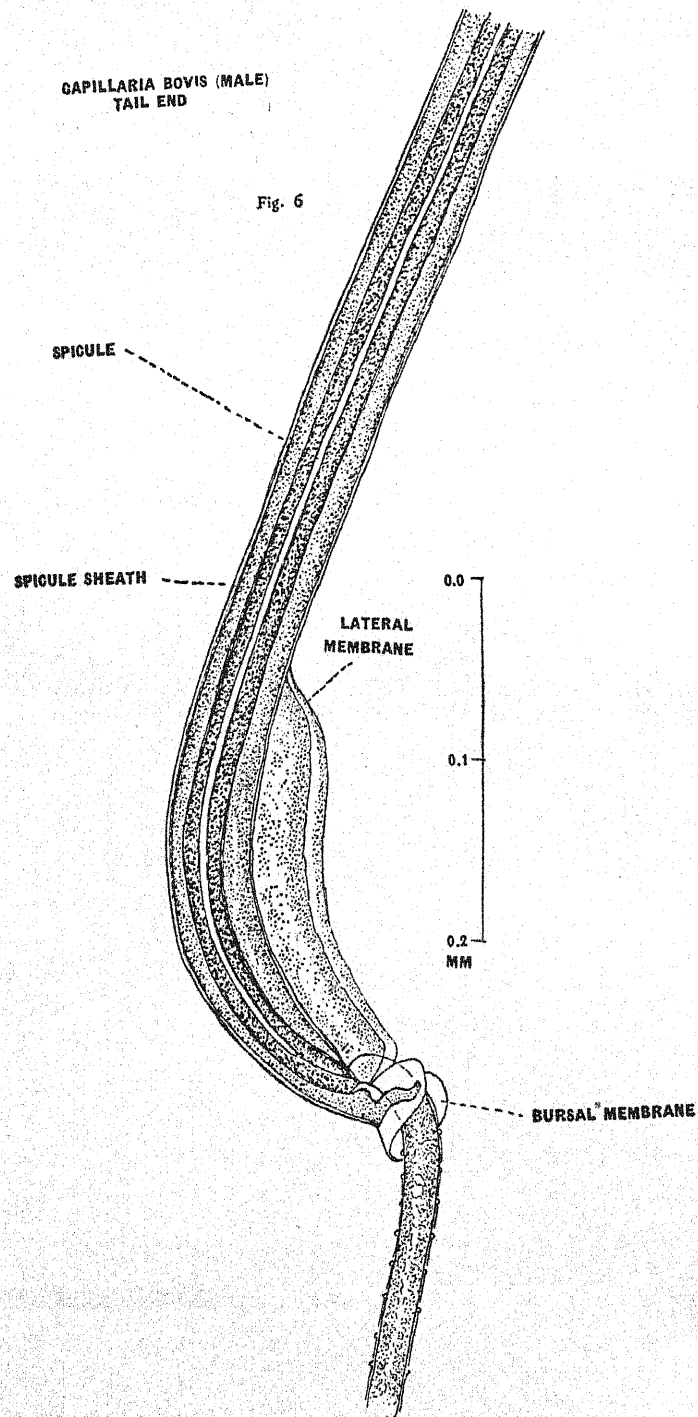
**LAST TWO CELLS OF THE OESOPHAGUS**

**Fig. 3**



**REGION OF THE VULVA**





Female is 21·4-21·8 mm. long and 0·088-0·097 mm. in maximum thickness. Oesophagus measures 7·00-7·25 mm. in length. The tail end is blunt (Fig. 4). There is only one ovary, which begins at the rectal region and extends anteriorly; it thins out, bends on itself for a short distance and continues anteriorly again as a broad uterus filled with eggs. The uterus passes into a muscular vagina (Fig. 3) which opens by the vulva near the termination of the oesophagus, 7·20 mm. from the head end. The muscular vagina measures 0·220 mm. Eggs are barrel-shaped (Fig. 5) with plugs at each end containing when deposited an unsegmented ovum measuring 0·047 mm.  $\times$  0·026 mm.

Male is 11·8 mm. long and 0·065 mm. in maximum thickness. The oesophagus is 4·50 mm. in length. The single testis which is bulky occupies most of the posterior region. Spicule (Fig. 6) is single, long and measures 1·01 mm. Sheath of the spicule is without spines and longer than the spicule when extruded. Tail end is curved ventrally. Along each side of the posterior end of the body beginning about 0·250 mm. from the tip of the tail, a rather broad membranous expansion extends as far as the anus. There is, besides, a membranous buras-like structure at the tip of the tail supported on each side by a leg-like process.

#### ACKNOWLEDGEMENTS

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# A NATURAL CASE OF CUTANEOUS LEISHMANIASIS IN A BULLOCK IN ASSAM

BY

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(With Plates VI to IX)

THE infection of domesticated animals with leishmania parasites has been recorded several times. In countries where leishmaniasis has been investigated upon, both visceral and cutaneous forms of the disease have been observed, either as separate disease entities or in combination, affecting both man and animals. Among the lower animals the cutaneous form of the disease has been investigated in some detail in canines [Mills, MacHattie and Chadwick, 1930], and its occurrence has been reported in cats [MacHattie, Mills and Chadwick, 1931], in a horse [Bennett, 1935] and in a bear [MacHattie, 1927].

In India our knowledge of leishmania infection of animals is limited to few cases among dogs [Avari and Mackie, 1924; Row, 1925; Sinton and Shortt, 1934] indigenous to the western part of the country where Oriental sore, locally known as Delhi boil or Lahore boil, occurs as a commonly recognised human infection. The present communication deals with this disease in a bullock. This is considered to have considerable scientific interest, firstly, because leishmaniasis has never been described in a bovine host and secondly, as it relates to the occurrence of the disease in an animal other than the dog in an important endemic centre of kala-azar.

## HISTORY OF THE CASE AND DESCRIPTION OF THE LESIONS

While working as the Veterinary Investigation Officer for Assam, the writer in December, 1933, encountered a case of cutaneous leishmaniasis in a bullock in the village of Naura-Kuwar situated in the Golaghat sub-division which for the past few years has been known to be an endemic centre of human kala-azar.

The subject was an aged bullock purchased from a wandering cattle dealer who had brought his animals by road from another town in Assam for sale in this sub-division. The owner stated that at the time of purchase the animal had several nodular lesions on both the hind legs extending from the hock to the fetlock, and that similar lesions were observed on the feet and tail of a few others of the batch. No information could be obtained as to where the rest of them were disposed off. The owner also gave the information that the nodules were transformed into sores about two months after the purchase of the animal. The condition as observed at the time of investigation was stated to have been persisting for the previous nine months. The animal suffered from no systemic disturbance, but it showed as a result of neglect signs of general emaciation and unthriftiness presumably. It

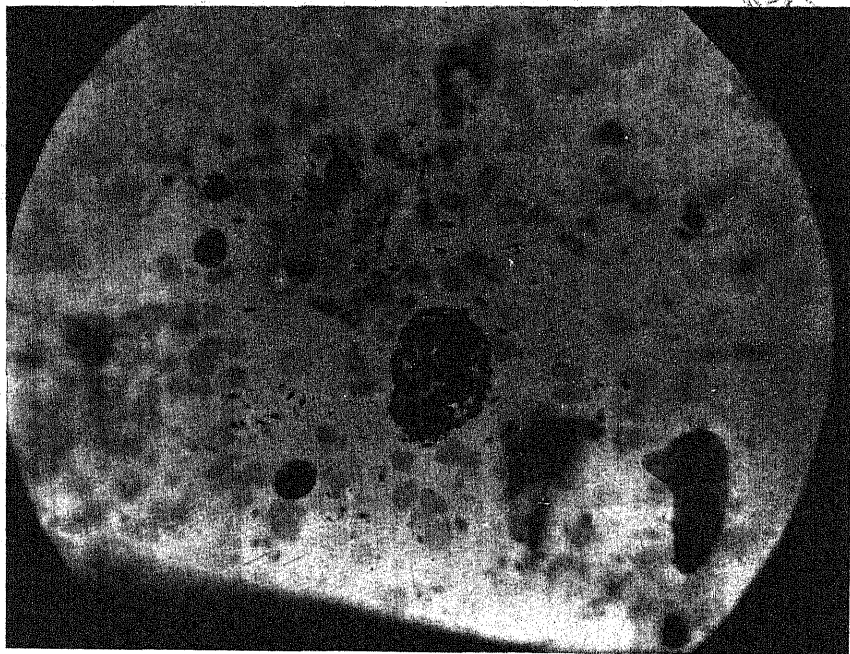
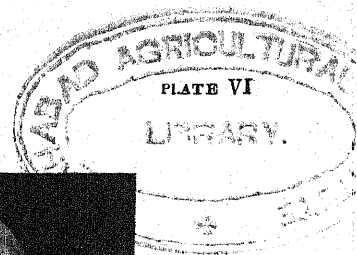


FIG. 1. Leishmania bodies occurring free as well as inside a macrophage in the tissue smear  $\times 500$

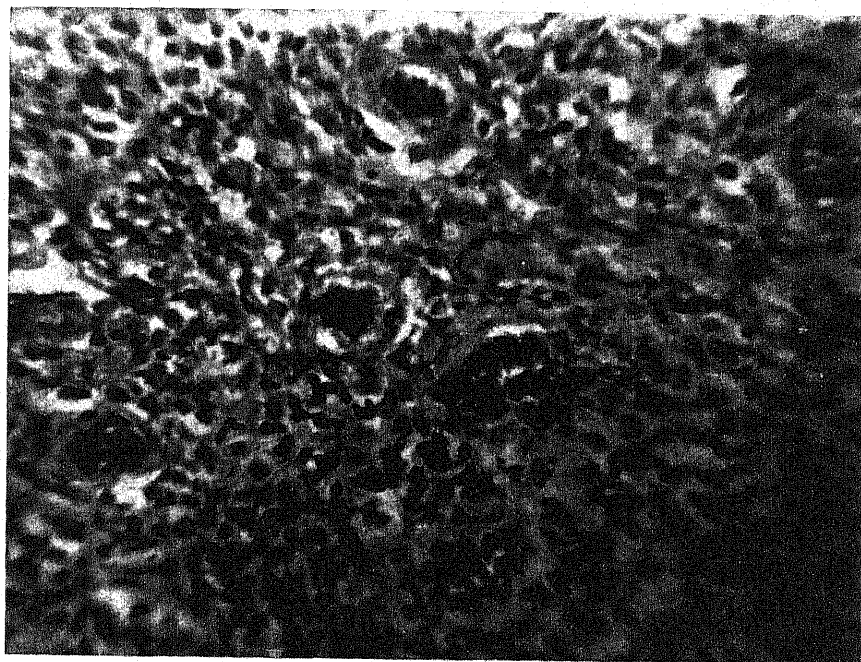


FIG. 2. A compact cellular mass of granulation tissue associated with a number of partially or completely occluded blood vessels  $\times 450$



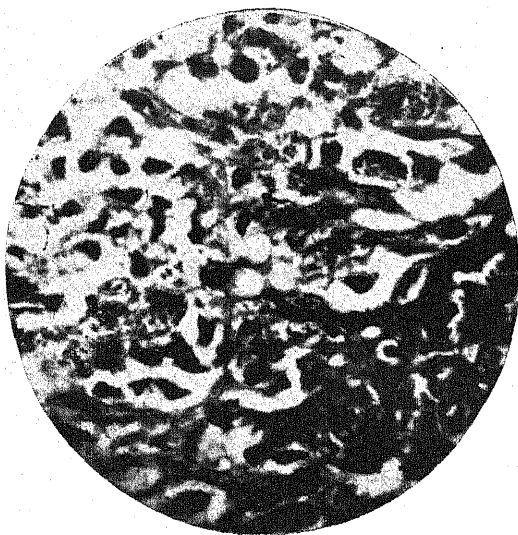


FIG. 1. Macrophages loaded with leishmania bodies disposed in the vicinity of dilated lymphatics  $\times 500$

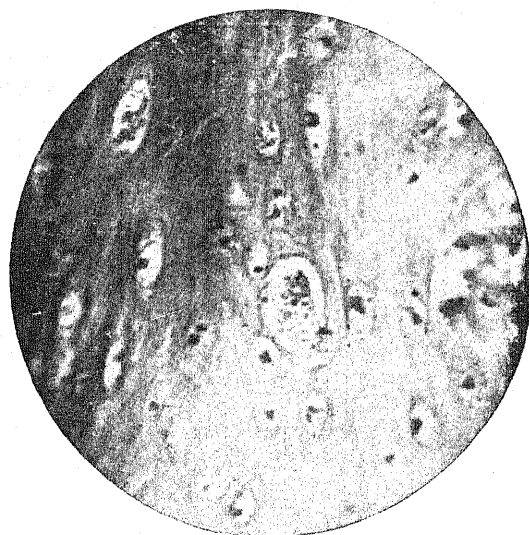


FIG. 2. An area of degeneration with a few cellular elements and macrophage containing leishmania bodies  $\times 880$



FIG. 3. Ulcerated part and downward growth of the *rete malpighi* together with the transformation of this layer in isolated masses  $\times 150$



FIG. 4. Downward growth and branching of the *rete malpighi* and the presence of nest cells  $\times 46$

showed an enlargement of both prescapular lymphatic glands and an infestation with the ticks, *Hyalomma aegyptium*.

The village in question was visited twice at an interval of about a year. On the second visit no fresh cases of the disease were seen, presumably, as the infected animal was kept in a field at a distance from where other animals were located, for the period it remained with the owner.

In the affected bullock the following two types of lesions were recognised :—

*Circumscribed ulcerative type.*—Only two lesions of this type were found. These were situated on the inner aspect of the fetlocks of the hind legs. About half an inch around the ulcers the hairs were found to be broken and scanty. The lesions measured about an inch in diameter. The peripheral parts consisted of heaped-up granulation tissue presenting a smooth, shining and highly congested appearance. The floors of the ulcers were depressed and contained a crumbling necrotic mass. Excision of the lesions at the first visit was followed in about a year by new formation of tissue to the size of the original growth. The excised surface of the tissue appeared oedematous and showed downward growth of the *rete malpighi*.

*Diffuse ulcerative lesion.*—This kind of lesion was found extending continuously from the hock to the pastern of the right leg. The lesion had no sharply defined margin and presented a raw surface covered with an exudate hardened at places into crusts which could be readily removed. This peculiar appearance of the lesion may be ascribed to the confluence of several smaller lesions by rupture and the consequent peripheral extension of the ulcerated part of the skin in this area.

#### MICROSCOPICAL DIAGNOSIS

*Blood.*—An examination of the blood taken from the ear vein revealed 7,500,000 red blood corpuscles and 5,800 leucocytes per cubic millimeter as estimated by Gower's haemocytometer. Leucopenia was thus present. The haemoglobin content of the blood was estimated to be 60 per cent of the normal. On a rough estimate coagulability seemed to be markedly increased. Differential leucocyte counts on the blood from the ear vein and from the peripheral part of the lesion showed the characteristic differences noted below.

TABLE I

*Differential leucocyte count in blood films from the ear and the peripheral part of the sore*

Leucocytes	Blood from ear	Blood from sore periphery
Neutrophiles	(per cent) 28·6	(per cent) 15·4
Eosinophiles	14·8	2·5

Leucocytes	Blood from ear	Blood from sore periphery
	(per cent)	(per cent)
Basophiles	0·20	0·20
Monocytes	4·8	25·8
Large lymphocytes	20·5	52·6
Small lymphocytes	34·2	20·2

The above table shows a marked increase of monocytes over neutrophils, in the smears obtained from the periphery of the sore. The total count of the large and the small lymphocytes in these smears is greater than normal, and the former variety of lymphocyte is more numerous than the latter. These haematological findings are similar to those obtained by Cardamatis [1909] in several cases of Oriental sore in Greece.

*The tissue smears.*—The sites selected for making tissue smears for microscopical examination were the margin of the crater-like depression of the nodular growths and the ill-defined borders and the surface of the diffuse type of the sore. The smears were fixed and stained by Leishman's method. Numerous leishmania parasites, mostly extra-cellular, were seen although a fair number of mononuclear leucocytes or endothelial cells contained these organisms in clusters in their cytoplasm (Plate VI, fig. 1).

The mature forms of the parasite, unlike those of the human Oriental sore, were either pyriform or spindle-shaped. They measured 4 to 5 microns in length and 2 to 2·5 microns in breadth. The immature forms, commonly seen inside the cytoplasm of the mononuclear cells, were rounded or globular in shape, either with or without a vacuole in the centre. The tropho and the kineto-nuclei, considered to be characteristic of leishmania bodies, were situated in the widest part of the mature parasites. Thus they occupied a central position in the spindle-shaped forms and the basal region of those with a pyriform appearance. In the majority of the parasites the kineto-nuclei were situated tangentially anterior to the tropho-nuclei, but in some they occupied a position parallel to the longitudinal axis of the parasites while maintaining the tangential position. In short, the morphological characters of the parasites did not differ essentially from those of *L. donovani* or *L. tropica* of human origin.

#### HISTOPATHOLOGY

The nodular growths on the fetlocks were excised and fixed in 5 per cent formol saline. Sections were stained in various ways, the best results being obtained with Heidenhain's iron haematoxylin and eosin. The lesions,

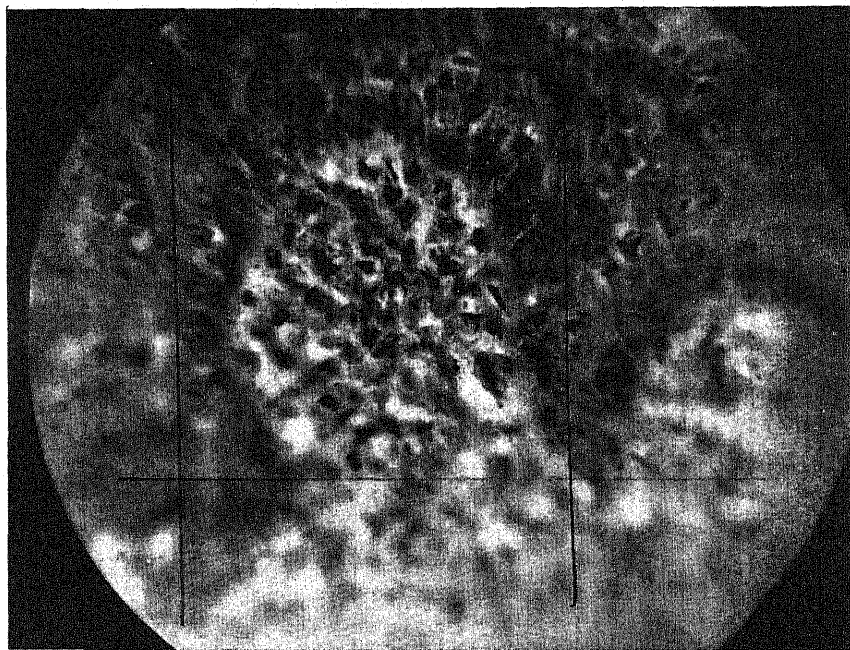


FIG. 1. Macrophages in the papillary region of the corium, containing leishmania bodies  $\times 500$

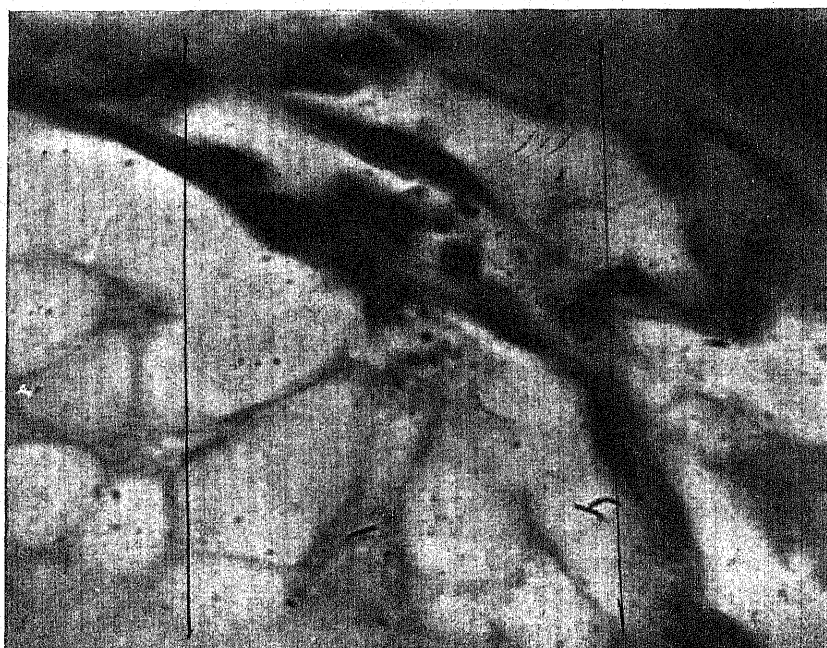


FIG. 2. An endothelial cell of the inner lining of a dilated blood vessel containing the leishmania bodies  $\times 1,000$





FIG. 1. Proliferation of the endothelial lining of a blood vessel. Note one of these cells containing the leishmania bodies  $\times 1,000$

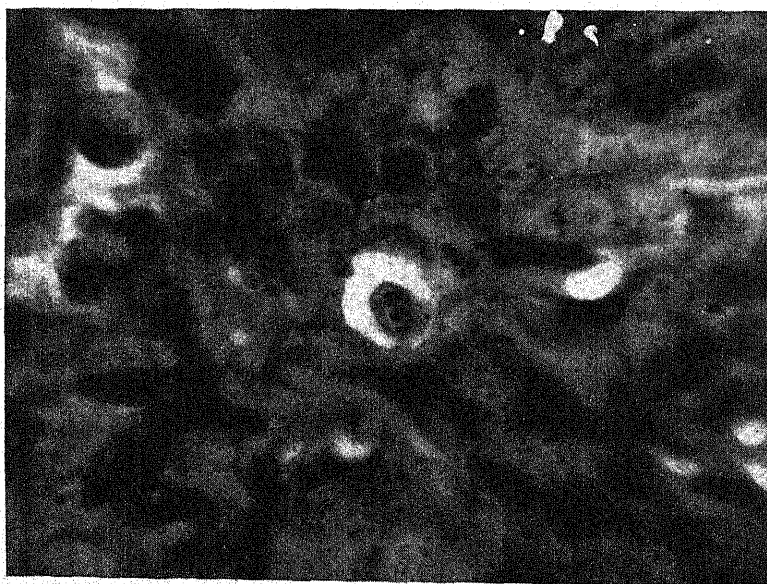


FIG. 2. Two leishmania bodies occurring freely in the lumen of a lymph vessel  $\times 1,000$



which had persisted for two years, were at an advanced stage and earlier changes could be seen in the peripheral region. At the centre there was granulation tissue (Plate VI, fig. 2) and in its centre the parasitized endothelial cells were few and not readily detectable, while the parasites stained feebly and were ill-defined. A greater part of this region revealed a sero-fibrinous type of inflammation resulting in dilatation of the lymphatic channels (Plate VII, fig. 1) and the formation of loose cellular matrix due to diapedesis of lymphatic and other inflammatory cells in that region. Columns of endothelial cells or the macrophages in this region were found disposed in the vicinity of the dilated lymphatics and contained leishmania bodies in their cytoplasm. The process of cellular degeneration at certain places in this region resulted in the formation of structureless masses containing only occasional cells (Plate VII, fig. 2) including parasitized macrophages.

Histological examination of the superficial part of the sore in the central region revealed the solution of continuity of the epidermis (Plate VII, fig. 3), whilst the peripheral region was characterised by an intense cellular infiltration and the congestion of blood vessels in the papillary portions of the corium. The most important finding in this region is the presence of numerous nest cells regarded by Lazarus-Barlow as characteristic of this condition (Plate VII, fig. 4). The *rete malpighi* layer is hypertrophied, grows downward and branches into the underlying corium. The cells of this layer show marked perinuclear vacuolation. The papillary region shows excessive proliferation and hypertrophy leading to encroachment on and disintegration of the *rete malpighi* which as a consequence becomes transformed, to a certain extent, into isolated masses of cells (Plate VII, fig. 3) lying in the corium. The papillary part of the corium shows an extensive invasion by macrophages (Plate VIII, fig. 1) containing numerous leishmania parasites. In the corium proper and in the vicinity of sebaceous and sweat glands accumulations of macrophages are found near blood vessels some of which were occluded with proliferated endothelial cells. Careful examination of the dilated blood vessels in this region revealed a few intimal cells (Plate VIII, fig. 2) containing the causative micro-organisms. Proliferated intimal cells occluding the lumen of the smaller blood vessels (Plate IX, fig. 1) have also been observed containing the parasites, and the macrophages considered as typical of leishmania infection may thus be regarded as proliferated endothelial cells. The parasites, besides occurring intra-cellularly, have also been observed lying free in the lumen of a lymph vessel (Plate IX, fig. 2) and in tissue spaces mixed with inflammatory cells.

Our observations on the histopathology of this disease in the bovine host agree closely with the published accounts of leishmaniasis in man by Manson [1907], Wright [1903], Brooke [1903] and Balfour [1917] and that in canines by Mill, MacHattie and Chadwick [1930]. Contrary to the observations of Jeanselme and Rist [quoted by Balfour, 1917] on the histopathology of 'Bouton d'orient' focal necrosis and giant cells formation were not seen in our bullock.

#### DISCUSSION

In India there are two types of leishmania infection, firstly, the visceral form caused by *Leishmania donovani* and known as kala-azar principally

affecting man, and secondly, the cutaneous infection known as Oriental sore due to *Leishmania tropica* affecting both man and the dog. As a result, however, of the researches of Laveran and others [1917] the parasites of both forms of the disease have been shown to be immunologically different, although inoculation of *L. donovani* into laboratory animals, besides producing visceral leishmaniasis, may also produce lesions characteristic of *L. tropica*, and *vice versa*. Morphologically, no characteristic differences have been observed between these two species, and the parasite observed in the present case bears a close resemblance to the two known species.

Work on the specific identity of the parasite could not be undertaken due to lack of laboratory facilities in Assam. However the epizootological facts known regarding both forms of the disease as prevalent in India are worthy of consideration. The geographical regions in which one form of the disease prevails are entirely distinct from those in which the other occurs. Thus according to Sinton [1925] only *L. tropica* infection prevails, both in man and dog, in the western part of the country or in the region north-west of a line joining Delhi and Bombay, while in parts south-east of this line only *L. donovani* occurs. Further, the commonly recognised insect vector of *L. tropica*, according to workers at Calcutta School of Tropical Medicine [Knowles, 1928], is *Phlebotomus sergenti*, and, as Sinton [1925] has observed, there is a co-relationship between the geographical distribution of *P. sergenti* and of Oriental sore. Sinton records that he has never found *P. sergenti* amongst sandflies collected in places south and east of a line joining Bombay and Delhi; also that Oriental sore caused by *L. tropica* does not occur south and east of this line. Although experimental infections with *L. tropica* have been successfully set up with some certainty in dogs and monkeys, they have failed in the hands of Chadwick and MacHattie [1927] in such animals as horses, cattle, sheep, a pig, fowls and rabbits.

The available epizootological and experimental data make it very doubtful if *L. tropica* happened to be the cause of the sores in the present case. The possibility of *L. donovani*, being involved in the present case, has to be judged in the light of the following established facts concerning this species: (a) the occurrence of *L. donovani* south-east of the line joining Bombay and Delhi, and the non-occurrence of a single case of Oriental sore caused by *L. tropica* in man in this province, (b) the common insect vector of *L. donovani*, *Phlebotomus argentipes*, is found abundantly in endemic centres of kala-azar in Assam and Bengal where according to Lloyd, Napier and Smith [1925] the fly prefers to feed on cattle rather than on man, (c) both in experimental animals [Row, 1912, 1913 and 1914] and in man *L. donovani* may cause cutaneous lesions. In post-kala-azar cases [Brahmachari, 1922] and in those having no previous history of kala-azar [Acton, 1926] dermal leishmaniasis of various clinical types has been encountered and described. Napier and Dasgupta [1934] and Smith and Haldar [1935] have described ulcerative lesions in man in which *L. donovani* have been found.

The present case of ulcerative dermal leishmaniasis in a bullock in Assam would thus appear to be strongly suggestive of a *L. donovani* infection. Although only one case of this disease in a bovine is being described here

it is believed that others will be detected in endemic kala-azar centres in Assam, if and when specially looked for.

The close relationship of canine leishmaniasis to infantile kala-azar in countries on the Mediterranean littoral had caused workers in this country to suspect that dogs indigenous to endemic centres of kala-azar might suffer from the natural infection. For instance, Shortt, while working in Assam in 1923 during the height of a kala-azar epidemic, examined 44 dogs showing ill-health and emaciation for leishmania infection, but the results were negative. Unfortunately dogs alone were suspected of being naturally infected, and other domestic animals living in association with man were not similarly examined. In conclusion, the record of this single case of bovine leishmaniasis in Assam, taken in conjunction with the natural habits and bionomics of *P. argentipes* which is associated with cattle in this province, indicates that the epizootology of Indian kala-azar requires further investigation as regards the question of natural infection in animals.

#### SUMMARY

A clinical and histopathological description is given of cutaneous leishmaniasis in a bullock from an endemic kala-azar centre in Assam. The organism involved in this case may be *Leishmania donovani*.

#### ACKNOWLEDGEMENTS

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## SMUGGLING OPIUM AND CHARAS IN THE STOMACHS OF CAMELS

BY

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IN the *Excise Supplement of the Bombay Police Gazette* of 1 August 1940 is published an interesting extract from the Annual Report for 1939 of the Central Narcotics Intelligence Bureau, Cairo, Egypt, on smuggling opium and charas into Egypt in the stomachs of camels.

About 30,000 to 35,000 camels are driven into Egypt annually from the East for the meat markets of the Nile valley. In October 1939, information was received at Rafa that certain persons were going to run narcotics through Sinai concealed in the stomachs of camels which were to be driven through, ostensibly for the meat markets. This information was at once passed on to El Arish and Kantara where certain persons driving their camels across Sinai were arrested.

Some difficulty was experienced in determining which camels were carrying the narcotics owing to the large number of camels arriving at Kantara from the East at that time. However 'the Sinai Police are an exceptionally subtle body of men and can almost smell narcotics through a brick wall' and eventually nine camels were put under suspicion at Kantara and three others were soon in the lock-up at El Arish. One of the grounds for suspicion was that the owners refused £E 10 for a miserable camel not worth £E 3.

One of the camels detained at El Arish was slaughtered and in the rumen were found 27 containers with conical ends each 15 cm. long and 4 cm. in diameter. This information was conveyed to the *Mamour* of Kantara with instructions to slaughter the nine camels detained there while the two remaining camels at El Arish were also slaughtered and found to contain further narcotics. At Kantara, the nine camels had been released but were soon rounded up again and every one was found to carry narcotics in similar containers. From eighteen camels seized 17·770 kilos of hashish and 62·593 kilos of opium, worth about £E 2,200 in Egypt, were recovered.

It is pointed out that 'An interesting feature of this case is the strange ability of the camel to swallow 25 heavy containers or cylinders 15×4 cm. and weighing 250 gm. and to be able to travel and work with little or no inconvenience to himself.'

'The camel is a ruminant and chews the cud ; to prevent these containers being regurgitated they were weighted inside with a certain quantity of lead ; they were also made too large to pass from the rumen or first stomach into the second and other stomachs. The rumen has at the sides a number of sacks in which the camel carries his water ; it is in these sacks that the cylinders lodged up. According to the veterinary authorities the rumen possesses



little or none of the digestive functions of the other stomachs and foreign objects such as these containers might lie there for weeks without upsetting the camels digestion, especially when made of zinc and not tin. They are also carefully soldered up to prevent any action on the contained drugs from the heat or acids of the stomach. Presumably a camel so loaded would eventually lose condition and die, but in the present case the poor beast has served his purpose if he has managed to carry the tins for the six or seven days needed to travel from Khan Younis to West of Kantara.' The containers are forced down the camel's throat, presumably in the same way as a 'ball' is administered to a horse.

Since the number of animals that pass through the quarantine stations is so large and it will not be possible to rely on information in future, it is stated that steps are being taken to apply scientific methods for the detection of the containers and each station will have to be equipped with an X-Ray or other similar apparatus and a certain proportion of the camels submitted as a routine measure to 'its searching beam.'

An interesting case of the use of science in the perpetration of crime as well as in its detection !

# AUTHORS ON INDIAN VETERINARY SCIENCE: THEIR WORKS, AGE, AND ANTIQUITY

BY

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(Received for publication on 10 January 1941)

## INTRODUCTION

THERE is an erroneous notion, generally prevalent among Western readers and Indians educated on European lines, that the ancient Hindus were a race devoted only to metaphysics and religion and that they never took any active, practical and scientific interest in the life of the world around them. But this is not really the case. In the early days of Indian history, though metaphysics and religion were indeed India's greatest contribution to world thought, other arts and sciences were not neglected but rather were developed with great precision and interest.

Generally, so-called modern history begins where the real history of ancient India ends. The birth of Lord Buddha and the beneficent reign of the great Asoka mark not only the beginning of modern times but the ending of India's 'Golden Age.'

This country known as Aryavarta or the abode of the Aryans was a store-house of learning for the whole world, and it may not be an exaggeration to say that, in every branch of science in ancient India, in the words of Captain P. Johnston Saint of the Wellcome Historical Medical Museum, 'even the most irreverent layman would see the distinct forerunner of all the modern marvels of today'. Indeed Indian literature written some thousands of years ago has actually indicated lines for research of a most up-to-date character.

The Hindus were among the first to develop many aspects of astronomy and eminent persons such as Cassini, Bailly, and Playfair are of the opinion that the observations and conclusions of Hindu astronomers of some thousands of years before Christ are of real aid in the development of this science to-day.

In the field of mathematics, some historians are of the opinion that the credit generally given to Pythagoras is really due to the ancient Indian mathematicians from whom he borrowed his theories. Hindu law based upon the writings of Manu of some hundreds of years before Christ, Yajna Valkya, Parasara, Brihaspathi, Sukra and others is still the basis of the law for Hindus in India. So also in the field of medicine, India held a prominent place in ancient days and evidence has been produced by Pocock, Dietz and others to show that the ancient Egyptians, Romans and Greeks owed more than is generally recognised to ancient India.

In his previous articles on 'The veterinary science in India, ancient and modern, with special reference to tuberculosis' and 'Veterinary surgery and surgical instruments of ancient India' (published in the November 1937 and January 1939 issues respectively of *Agriculture and Livestock in India*) the

the author has already traced the birth, growth and general development of the medical and veterinary sciences in all their aspects in ancient and medieval India. Again in another article on the 'History of animal husbandry in ancient India' the author [Krishnaswamy, 1941] has tried to show how the early Aryans in India were a race of agriculturists depending solely upon agriculture and livestock for their livelihood, and how on account of the various uses to which the animals were put, and the several animal products that were in use in those days, the early Aryans regarded animals as their foremost care. It is no wonder then, that in common with all other sciences, veterinary science developed to a high level, and several manuals were written regarding the proper management of domestic animals. For the purpose of this article, the writer is concerned only with authors on Indian veterinary science, their works and their antiquity.

#### WRITERS ON INDIAN VETERINARY SCIENCE, THEIR WORKS AND AGE

Among the original and authentic authors on Indian veterinary science are found the names of Sālihōtra, Pālakāpya, Rājaputra, Vaisampāyana, Vyāsa, Nakula, Sahadēva, Garga, Mrigasarma, Brihaspathi, Nārada, Gana, Jayadatta Sūri, Dinapathi, Malladēva Panditha, Simhadatta, Nala, Vātsya, Sukra, Manu, Kāutilya, and Parāsara; and among the authors of later date come Jayadēva, King Indusēna, Bhōja, Sārangadhara, Sōmēswara, Vāhada, Basavamantri, Gēērvana yuddhavikrama, Viswanāth Vajpēye, Sivamara Bhūpathi, Dipankara, and the poet Rudradēva. Each one of the above authors is reported to have contributed a valuable book on veterinary science; but many of these works are now lost either in part or completely; but some fragments of a few of them are still available here and there, in libraries and oriental institutions, where old palm-leaf manuscripts are preserved. Of all the authors mentioned above, the names of Sālihōtra as the authority on horses, and Pālakāpya on elephants stand pre-eminent. The exact period when these sages lived is difficult to determine, but evidence goes to show that they lived in a very remote age.

*Age of Sālihōtra.*—Sālihōtra is said to have lived in Salutār, a country near Gāndhāra, the modern Kāndahār. In the *Linga Purāna* (Ch. 7 and 24), among the different *Yogāchāryās* and their disciples, flourishing in the *Varāha Kalpa* of the present *Vivasvata Manvantara*, are given the names of both Sālihōtra and Agnivēsa, as having been the disciples of the same *Yogāchārya*, in the Neimisāranya land of the present 28th *Kaliyuga*. This points to Sālihōtra's having lived in a very remote age.

In one of the manuscripts entitled *Asvāyurvēda Siddhayōgasangraha* (P. P. S. No. 11251 and Burnell's Catalogue No. 12302, S. M. Library, Tanjore), the authorship of which is ascribed to Sālihōtra, the author describes himself as the son of the Sun God, as having learnt the science of horses from Brahma himself and as having taught it to his disciples. Elsewhere, in another manuscript, Sālihōtra is described as the son of one Hayaghōsha. In the latter, which is in the form of a dialogue between Sālihōtra and Susruta, Susruta is described in some places as the third son of Sālihōtra, and in others as his

disciple. In the eighth or the last part of a book called *Sālihōtra* in the Madras Oriental Manuscripts Library, Susruta is described as the son of Sālihōtra. In the *Mahābhārata*, Susruta is described as the son of the sage Viswāmitra ; other evidence also leads us to assume that Viswāmitra was, indeed, the father of Susruta, and that Sālihōtra was only his teacher, and addressed him as his son in the same way as a teacher may address his disciple as his son. Though it is not our purpose now to elucidate these questions, it is clear that Sālihōtra was the teacher of Susruta, who is considered to be the father of Hindu surgery and whose work, the *Susruta Samhita*, has been proved by Professor Dietz to be of considerable antiquity.

The antiquity of Sālihōtra can again be inferred from the fact that he has been quoted by Hāemādri in *Vratākānda*. Some portions from his work have been quoted in the *Agni Purāna*, the *Matsya Purāna*, and the *Garuda Purāna*, and this goes to prove that Sālihōtra's work is pre-Purānic.

Sālihōtra is again mentioned by Sarvānanda in his commentary called *Tēekasarvasva of Nāmalingāsūsāsana*, the dictionary of Amarasimha.

Moreover, many other authors on horses, who are equally antique and authentic, begin their writings by paying respect to Sālihōtra, as the originator of the science. All this goes to prove that Sālihōtra is the foremost and indeed ' the father ' of all Indian authors on veterinary science.

His work on horses appears to be a very comprehensive one, consisting of eight parts (16,000 *slokas* in 120 chapters) dealing with practical farriery. It is a complete guide to the science of horses, dealing with their breeding, training, feeding, watering, stabling, and grooming, their care in health and disease, with an elaborate description of several diseases they are subject to, and their treatment. The entire work is not extant. A portion of it is, however, available at Tanjore, and other portions in stray parts are reported from Calcutta, Lucknow, Baroda and Nepal.

*Age of Pālakāpya*.—Next in importance comes Pālakāpya, the first and the most ancient author on elephantology. About the date and authenticity of his work, there appears to be some controversy. Burnell in his catalogue says that Pālakāpya's *Haṣṭhyāyurvēda* is no doubt a very modern compilation, even later than the *Sārasaṅgraha* on horses. No reasons, however, have been assigned by him for his conclusion. But Edgerton, Professor of Sanskrit, Yale University, U. S. A., remarks in the introduction to his book *The Elephant Lore of the Hindus* : ' All known texts agree in attributing the founding of scientific elephantology to a mythical sage, Pālakāpya. They likewise agree in making him reveal this elephant lore to an apparently mythical Rōmapāda, King of Angās, whose name is not otherwise known, etc. '. The last portion of the remark, viz. that the name of the mythical Rōmapāda, King of Angās, is not otherwise known, is one which is not borne out by facts. The name of Rōmapāda, King of Angās, occurs in the *Bālakānda* of the great epic *Rāmāyana*, where it is stated that King Dasaratha had invited to Ayōdhya, the sage Rishya Sringa, the son-in-law of Rōmapāda, King of Angās. It is therefore clear that Rōmapāda was a contemporary of King Dasaratha, and that Pālakāpya's book on elephantology must be even older than the *Rāmāyana*, as it is clear from the context in the *Rāmāyana* that King Dasaratha sent a deputation to Rōmapāda, King of Angās, before the birth of Lord Rāma. This points to the fact that the age of Pālakāpya and his work is the early epic period. Again in the ninth *skanda* of *Bhāgavatha*, we find a reference to



King Rōmapāda. Moreover, in the introductory chapter of Pālakāpya's *Haṣṭhyāyurvēda*, a conference of several sages invited by Rōmapāda is mentioned, and among the guests which include Pālakāpya, we find the names of such sages as Agnivēsa, Bhāradwāja, Viśwāmitra, and Vasishtha whose antiquity has been beyond all doubt settled. As already pointed out by me elsewhere, Agnivēsa was a contemporary and co-disciple of Sālihōtra under the same *Yōgāchārya* in the Neimisāranya land in the present 28th *Kaliyuga* of the *Vivasvatha Manvantara* of *Varāha Kalpa*. Besides, in a manuscript copy of *Brihaj-yothishārṇava*, which I accidentally came across, and whose date has not been fixed, I found a passage which, translated, shows that Pālakāpya taught the *āyurveda* of elephants to the King of Angās. In addition, we find in the *Agni Purana*, copious quotations from Pālakāpya's *Haṣṭhyāyurvēda*. The antiquity of Pālakāpya can again be inferred from the following note in Afrechi's *Catalogus Catalogorum*, Vol. I (1890): "Pālakāpya is quoted by Kshēeraswāmi in *Amarakōsha*, Haēmādri in *Vratākānda*, *Sārangadhara-paddhati* (p. 90) and *Mallinātha*." Most important of all, Susruta, the father of Hindu surgery, is reported in some places to have learnt his art from Dhanvantari, the Vedic father of medicine, and in certain other places from Pālakāpya. This controversy as to whether Dhanvantari or Pālakāpya was the real teacher of Susruta has been reconciled by some eminent scholars, by assuming that both Dhanvantari and Pālakāpya are one and the same person. It is not our purpose to take part in this controversy; but it certainly goes to show that the sage Pālakāpya also belongs to a very remote past. Further, in an earlier part of this article, it has already been mentioned that Susruta was the disciple of Sālihōtra. From this it must be conceded that both Pālakāpya and Sālihōtra may have been contemporaries.

Next comes the name of Rājaputra who is reported to be an author on elephantology. In chapter 24 of the *Matsya Purana* we find the first three *slokas*, the translation of which is as follows:—

"After a year, a handsome cherub boy shining like the twelve suns, wearing yellow raiment, and resembling the moon, was born from the womb of Tāra. He was master of all the sciences, and the author of a treatise on elephants. Being the son of the Moon King, he was known as Rājaputra (King's son) and was afterwards named Budha."

Besides in Pālakāpya's book on elephantology, in the first chapter, a conference of sages is mentioned, wherein the name of Rājaputra is referred to as one of those invited to the conference. From the above, it is evident that one Rājaputra or Budha was the author of a book on elephants and a contemporary of Pālakāpya, who belongs to the early epic period. Beyond this we are not able to say anything, as the book itself appears to have been lost, and as no trace of any old manuscripts thereof appears to be available. It is, however, to be hoped that a search among the manuscripts available in Kashmir, Nepal and amongst the Jains of Gujerat may yield fruitful results.

Nakula and Sahadēva are the next authors commanding our attention. They were the sons of Mādri, second wife of Pāndu. Nakula was taught by Drōṇa, the training and management of horses, and Sahadeva, the management of cattle (vide *Virāta Parva* of *Mahābhārata*). This goes to show that these authors flourished in the epic period of the Mahabharata. In the *Brahma Vivarta Purāna* mention is made of Nakula, as being the author of a



book called *Vaidyaka Sarvaswa* or 'All about medicines' and Sahadeva being the author of a book called *Vyadhisindhu Vimardana* or 'The cure of the ocean of diseases'. These two books are not now extant. But the book called *Aswachikitsa* or 'The treatment of horses' as written by one Nakula is now available to us in print.

Mrigasarma, Brihaspathi and Narada are the other persons whose names are prominent in connection with the authorship of works on veterinary science. All these were invited by King Rōmapāda to the conference of sages referred to, in the beginning of Pālakāpya's book on elephantology and they are therefore recognized veterinary authors of authenticity and antiquity, and contemporaries of Pālakāpya in the early epic age. The works of Mrigasarma and Nārada are not so far available to us, but the work of Brihaspathi, known by the name of *Brihaspatimata*, is to be found in the Government Oriental Manuscript Library, Madras, but is not in print.

Then come the authors known by the names of Vaisampāyana and Vyāsa. These two authors appear to be one and the same person, generally known by the name of Vyāsa. The Editor of the *Journal of Oriental Research, Madras* in his review of Edgerton's *Elephant Lore of the Hindus* in the April-June 1933 issue of the journal remarked that Vyāsa's work on elephantology was not so far available to him. But during my search among the manuscripts of the S. M. Library, Tanjore, in 1939, I found that the works on veterinary science reported to be from the pen of Vyasa and Vaisampayana, viz. the books on horses called *Aswāyurvēda Sārasindhu* or 'Treatment of horses' and the one on elephants called *Gajasāstram* or 'The science of elephants' both under the authorship of Vaisampayana and another book on elephants called *Gajalakshana Chikitsa* or 'The treatment of the ailments of elephants' under the authorship of Vyāsa are available there, but require detailed study. Besides, as Vyasa is acknowledged to be the author of several of the Hindu *Purānas*, it is likely that the copious information on veterinary matters available in the *Matsya*, *Garuda*, *Agni*, *Brahmānda*, and *Linga Purānas* is of his authorship.

Garga is the author of another work on the treatment of horses. Reference to Garga as an author on medical science is made in a book called *Prayōga Ratnākara* and in the *Matsya Purāna*. His work on horses was believed not to be available, but during my recent tour in Orissa in connection with the indigenous veterinary drugs enquiry scheme of the Imperial Council of Agricultural Research, I came across a palm-leaf manuscript of this work in the Ravenshaw College library at Cuttack.

Next in order come Gana, the author of a book on horses called *Aswāyurvēda Siddhayōga Sangraha* (now available at the S. M. Library, Tanjore, and Government Oriental Manuscript Library, Madras) Jayadatta Suri, the author of a work on horses called *Aswa Vaidyaka* (now available in print); Malladeva Panditha, the author of the book on horses called *Aswāyurvēda Sāra Sindhu* (now available at the S. M. Library, Tanjore) and Dinapathy, Simhadatta and Nala, whose works have not so far been traced. Though it is not possible for me to discuss in detail the age and authenticity of each one of these authors, frequent references to them and their works by later authors show that they and their original works are very nearly as old as those of the earlier authors dealt with above.

It is now evident that almost all the authors of works on veterinary science in ancient India can be classified as having flourished in one or the other of the following three periods :

(1) The Vedic period when the four Vedas were written. The Atharva Veda, which contains the treatises on medicine and also some information on veterinary science, belongs to this period.

(2) The Epic period, during the early part of which the *ayurveda* or the science of the knowledge of life came into existence. It is to this period that Sālihotra, Pālakāpya, Rājaputra, Mrigasarma, etc. the first and original authors on veterinary science belonged. In the latter part of this period, i.e. the age of the Mahabharata, Nakula and Sahadeva flourished.

#### THE PERIOD OF THE *PURĀNAS* AND *SŪTRAS*

The several *Purānas*, wherein copious information on veterinary matters is available such as the *Matsya*, *Garuda*, *Agni*, *Brahmanda* and *Linga Purānas*, as also the several books on polity wherein also much useful information on veterinary matters is found, such as *Manusamhita*, *Kautilya's Arthasastra*, *Bṛhaspathi Mata*, *Parāsarasmhita*, and *Sukranīthi*, all belong to this period.

Space does not permit me to deal with each one of the other writers mentioned above and to discuss his antiquity ; but from such portions of their works as exist, it is evident that they were all contemporaries of one or the other of the several authors who flourished in the above periods.

#### CONCLUSION

It has been made sufficiently clear in this article that some of the works on ancient Indian veterinary science which were believed to have been lost, are actually extant and may be found in one or the other of the libraries which I visited in the course of my recent tour in connection with the enquiry into indigenous systems of veterinary medicine financed by the Imperial Council of Agricultural Research. Even in the case of the other works which are not so far available to us, one cannot conclude that they are lost, and in the absence of a definite search we may hope that they still remain to be unearthed. In the case of other Indian sciences, there have been many pioneer workers who toiled hard in their fields of research and their labours have been rewarded by the publication of numerous ancient works. This has definitely been the case in the field of ancient Indian archaeology, mathematics, astronomy, astrology, medicine, religion, law, metaphysics and polity ; while in the field of veterinary medicine, there have been hitherto neither such pioneer workers nor any encouragement either from the public or the profession. It is only now that there appears to be a little general awakening and the Imperial Council of Agricultural Research has begun to take a definite interest in the subject. It only remains for me to say that a collection of all the ancient Indian veterinary literature now available and a systematic search for such works as have so far not been traced, would be of very great interest and advantage to the veterinary profession.

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# A PRELIMINARY REPORT ON A METHOD OF VACCINATION AGAINST RANIKHET DISEASE

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OWING to the urgency of the problem of control the authors think fit to publish the recent results of their work on vaccination against Ranikhet disease. In order that, where conditions make it practicable, the knowledge so far gained may then be applied by laboratories in a position to undertake the preparation of the vaccine to the protection of flocks under the somewhat restricted conditions which are at the moment possible.

The idea leading up to these experiments was developed from the reports of success on similar lines with foot-and-mouth disease [Schmit *et. al*, 1936] and from earlier unpublished work on rinderpest at this Institute.

The general principle involved is the adsorption of the virus on alumina gels which are subsequently treated with dilute formalin to destroy the unadsorbed virus. The adsorbed virus is then inoculated and it is presumed that this is gradually released in minimal quantities into the bird's system. The reaction is in practically every case symptomless and confers an immunity of sufficient degree to resist a test inoculation of 10-100 or more M.L.D. of virus between the 8th and 21st day after vaccination.

The duration of immunity at longer intervals has not so far been tested and, until this is done, it is considered advisable to enhance the primary immunity conferred by the vaccine by subsequent inoculation with fresh virus within the time suggested above.

The preliminary experiments indicate that for any given temperature the time limits within which the vaccine matures and within which it retains its efficacy after maturation are remarkably precise, but unfortunately at the present time the keeping power is of very short duration — approximately 24-48 hours — and the authors realise that this places serious difficulties in the practical application of this method of vaccination except in favoured localities.

## METHODS

The alumina gel used in these experiments was prepared in every case according to Sabin's [1932] modification of the method prescribed by Willstätter and Kraut and, as it is absolutely essential that the details should be carried out precisely, the procedure is quoted in full:—

"*Preparation of aluminium hydroxide gel.*—Alumina gel C was prepared essentially according to the method of Willstätter and Kraut excepting that

the centrifuge was used instead of natural sedimentation. An effective gel was thus obtained, the process requiring only 2 days as compared with 2 weeks or more in the original procedure. Distilled water must be used throughout; in the preparation of a large quantity of this gel, tap water was used once and a product of entirely different physical and adsorptive properties was obtained. The following is the procedure for preparing 250 c.c. of the gel:—

To distilled water at 70°C. sufficient concentrated ammonia is added to make a 4 per cent solution. A filtered solution of 50 gm.  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$  in 150 c.c. of water at 65°C. is rapidly poured into the ammonia, and the mixture shaken vigorously for 15 minutes. The gel is then centrifuged until a well-packed sediment is obtained; the supernatant water-clear liquid is poured off. The sediment is made up to 1000 c.c. with water, shaken and recentrifuged; this process is repeated five times. After the fifth decantation, 400 c.c. of 4 per cent ammonia at 70°C. is added to the sediment and the mixture shaken for 15 minutes. It is then centrifuged, the supernatant liquid poured off, and the sediment made up to 1000 c.c. with water. After shaking, it is again centrifuged. The supernatant fluid is water clear until about the ninth or tenth washing, when it becomes opalescent. When this stage is reached the gel is washed once more and after centrifugation and decantation, the sediment is made up to 250 c.c. with water and thoroughly shaken with glass beads. The gel is standardized by determining the quantity of  $\text{Al}_2\text{O}_3$  per c.c. which is accomplished by drying 5 c.c. in a crucible at 110°C., igniting, and weighing the residue. When the quantities given above are used, different preparations contain from 21-25 mg.  $\text{Al}_2\text{O}_3$  per c.c."

To 1 c.c. of this gel 1 c.c. of an M/15 solution of acid potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) in distilled water is added, in order to attain a suitable pH for adsorption (pH 6.6-6.8), and then 1 c.c. of the virus mixture. The whole is mixed and placed in the refrigerator for 2½ hours. At no time must the gel be frozen, otherwise it will disintegrate.

After this period the gel is again stirred and centrifuged at 3000 r.p.m. for 30 minutes. The supernatant fluid is discarded, the deposit is made up to double its volume with 1/1000 solution of formalin in distilled water and stored at a predetermined temperature until mature.

The source of the virus has in all cases been the spleens of birds taken on the 4th, 5th or 6th day after inoculation with stock virus (the inoculum has consisted of 1 c.c. of 1/100 suspension of spleen, liver and kidney, preserved in 50 per cent glycerine). By the 4th to 6th day the birds are either moribund or dead. It is probable that other tissues could be used for the preparation of vaccine but as it was desired to keep the virus content as constant as possible only one tissue (spleen) was employed. The tissue is first sliced with scissors and then ground as finely as possible in a mortar, broth being added gradually to produce a homogeneous suspension. This is then passed through muslin and a Whatman No. 42 filter paper. The filtrate is still markedly turbid. In the first test the proportion of spleen tissue to broth was one gram to 20 c.c., the M.L.D. of the filtrate from this mixture being less than 0.5 c.c. of a  $1 \times 10^{-5}$  dilution; in subsequent experiments the proportion was one gram to 4 c.c. broth, the M.L.D. being 0.5 c.c. of a  $1 \times 10^{-7}$  dilution.

No detailed experiments have yet been undertaken to ascertain the quantity of virus adsorbed but it has been shown that practically all the virus is in the centrifuged gel deposit. Probably, however, a portion of the virus is attached to particles of spleen and is eventually killed by the formalin.

#### EXPERIMENTAL

The first experiment (30-5-1940) included the inoculation of 6 fowls in groups of two with vaccine stored at room temperature (14°-16°C.) for 8 days. The final dilution of formalin varied in the three lots from 1/500-1/2000.

TABLE I

#### *First vaccine experiment*

Dose of vaccine	Concentration of formalin	No. of fowls	Reaction to vaccination	Reaction to test dose virus (1 c.c. of $1 \times 10^{-4}$ )
0.5 c.c.	1/500	2	<i>Nil</i>	1 died tenth day after test. 1 survived.
0.5 c.c.	1/1000	2	<i>Nil</i>	Both died 9th and 12th day after test.
0.5 c.c.	1/2000	2	<i>Nil</i>	1 survived. 1 died 8th day after test.

NOTE:—(a) The test dose was administered 15 days after vaccination and the controls (6) died from 3-8 days after the test inoculation.

(b) The virus used for test in the experiments, except where otherwise stated, was a dilution of the original virus suspension from which the vaccine was prepared. This virus was stored in the refrigerator until used.

In two groups one bird recovered after test and the deaths of the other birds were delayed and were probably due to spirochaetosis; this disease was definitely diagnosed in two of the four birds which died. The outcome of this experiment was considered encouraging and further preliminary experiments to obtain some indication of the virus content and its viability at different stages of the preparation of vaccine were undertaken. From these it appeared *inter alia* that the stock virus mixture can be maintained in the refrigerator for 21 days or longer without noticeable deterioration, the vaccine



mixture if placed in the refrigerator immediately after preparation can be similarly stored for 12 days prior to the period of maturation at a higher temperature; while at room or incubator temperatures the vaccine becomes completely inert in 6 to 14 days or earlier, according to temperature and the approximate period of maturation of the virus at room temperature was 8 days.

A further experiment (28-9-1940 to 3-10-1940) was then carried out in an attempt to define approximately the time limits for maturation and a suitable dose for practical vaccination.

TABLE II

*Second vaccine experiment*

Date	Dose of vaccine	No. of fowls	Reaction to vaccination	Result of test on 19-10-40 with 0.5 c.c. of $1 \times 10^{-6}$ dilution of stock virus
<i>Test of vaccine stored for 48 hours at 37°C.</i>				
28 Sept. 1940	0.5 c.c.	2	Nil	Both immune
	1 c.c.	2	Nil	Both immune
	2 c.c.	2	Nil	Both immune
<i>Test of vaccine stored for four days at 37°C.</i>				
30 Sept. 1940	0.5 c.c.	2	Nil	Both immune
	1 c.c.	2	Nil	Both immune
	2 c.c.	2	Nil	Both immune
<i>Test of vaccine stored for six days at 37°C.</i>				
2 Oct. 1940	0.5 c.c.	2	Nil	1 immune 1 died†
	1 c.c.	2	Nil	1 died* 1 immune
	2 c.c.	2	Nil	1 died* 1 immune

\*Positive for Ranikhet disease.

†Probably due to other causes.

Immune = No visible reaction.

Date	Dose of vaccine	No. of fowls	Reaction to vaccination	Result of test on 19-10-40 with 0.5 c.c. of $1 \times 10^{-8}$ dilution of stock virus
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*Test of vaccine stored for 5½ days at 14°-16°C.*

29 Sept. 1940	0.5 c.c.	2	1 died*	
			1 Nil	1 immune
	1 c.c.	2	Nil	Both immune
	2 c.c.	2	1 died*	
			1 reacted severely	1 immune

*Test of vaccine stored for 7½ days at 14°-16°C.*

1 Oct. 1940	0.5 c.c.	2	Nil	1 died* 1 reacted and recovered.
	1 c.c.	2	Nil	1 died* 1 immune
	2 c.c.	2	Nil	Both immune

*Test of vaccine stored for 9½ days at 14°-16°C.*

3 Oct. 1940	0.5 c.c.	2	Nil	Both immune
	1 c.c.	2	Nil	Both immune
	2 c.c.	2	Nil	Both immune

*Controls to test inoculation*

19 Oct. 1940	..	6	..	All died*
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\*Positive for Ranikhet disease.

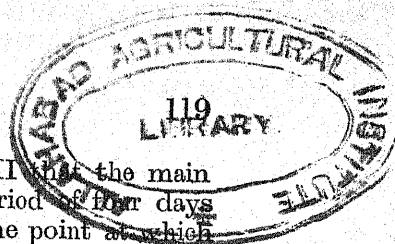
Immune = No visible reaction.

The intention up to this time was to devote attention more particularly to a vaccine prepared by the less energetic treatment by maturation at comparatively low temperatures. As, however, we had received an indication from earlier small-scale experiments, which need not be detailed here, that the alumina gel even without the addition of formalin had a distinct lethal action on the virus at room temperature, and that, therefore, the efficacy of the vaccine could not under the conditions of these experiments be preserved for long, it became apparent that speed in preparation would be an important factor if the vaccine was to be of any practical use in the treatment of outbreaks. Rapid maturation in the incubator was therefore attempted. It will be seen from the information given in Table II that after storage at 37°C. the vaccine was efficacious from the 2nd to the 4th day of inoculation, while with the material kept at room temperature (14°-16°C.) the results were irregular, those after 10 days storage being in accord with our previous experience. Several events prevented the continuation of any but small-scale experiments during the winter but another experiment on similar lines to the one already reported was taken up (26-2-1941 to 28-2-1941) and the results are given in Table III.

TABLE III  
*Third vaccine experiment*

Date	Dose of vaccine	No. of fowls	Reaction to vaccination	Result of test with 0.5 c.c. of $1 \times 10^{-6}$ dilution of virus
<i>Vaccine tested after 24 hours at 37°C.</i>				
26 Feb. 1941	0.5 c.c.	4	Nil	All immune
	1 c.c.	4	Nil	All immune
<i>Vaccine tested after 48 hours at 37°C.</i>				
27 Feb. 1941	0.5 c.c.	4	Nil	All immune
	1 c.c.	4	Nil	All immune
<i>Vaccine tested after 3 days at 37°C.</i>				
28 Feb. 1941	0.5 c.c.	4	Nil	All immune
	1 c.c.	4	Nil	2 died* 2 reacted and recovered.
<i>Controls to test inoculation</i>				
28 March 1941	..	4	..	All died*

\*Positive for Ranikhet disease.



It will be observed from the results given in Table III that the main objective approved to have been attained and that the period of four days which had given good results before was apparently near the point at which the vaccine loses its immunising value. To confirm this and to ascertain the infectivity of birds undergoing vaccination and to estimate the degree of immunity conferred a further batch of 35 fowls was inoculated with vaccine (20-3-1941 to 21-3-1941). The results are shown in Table IV.

TABLE IV  
*Fourth vaccine experiment*

Date	Dose of vaccine	No. of fowls	Reaction to vaccine	Test dose	Result
<i>Test of vaccine incubated 24 hours at 37°C.</i>					
20 March 1941	1 c.c.	10	1 died*	0.5 c.c. of $1 \times 10^{-6}$ dilution	All imm
			9 Nil		
	1 c.c.	1	Nil	1.0 c.c. of $1 \times 10^{-6}$ dilution	Died †
	1 c.c.	10	Nil	0.5 c.c. of $1 \times 10^{-4}$ dilution	1 died †, remainder immune.
	1 c.c.	4	Nil	0.5 c.c. of 1 in 20 stock suspension	All immune
<i>Test of vaccine incubated 48 hours at 37°C.</i>					
21 April 1941	1 c.c.	4	Nil	0.5 c.c. of $1 \times 10^{-6}$ dilution	1 died† 3 immune
	1 c.c.	4	Nil	0.5 c.c. of $1 \times 10^{-4}$ dilution	1 died † 3 immune
	1 c.c.	2	Nil	0.5 c.c. of 1 in 20 stock suspension	1 died † 1 immune
<i>Controls to test inoculation</i>					
4 April 1941	..	2	..	0.5 c.c. of $1 \times 10^{-6}$ dilution	Both died †
	..	2	..	0.5 c.c. of $1 \times 10^{-4}$ dilution	Both died†
	..	2	..	0.5 c.c. of 1 in 20 stock suspension	Both died†

\*Negative to Ranikhet disease.

†Died of Ranikhet disease.

It will be seen from this experiment that while the immunising value of the vaccine was not 100 per cent effective at 24 hours as in the previous experiment it still protects a very high proportion of birds. We are unable at present to explain the comparatively low rate of satisfactory immunisations in the lot receiving vaccine matured for 48 hours in the incubator. It will also be observed that the immunity produced is not always of such a high degree as to enable birds to resist a heavy experimental infection. It is probable, however, from a further experiment which will be reported in detail later that vaccinated fowls would resist a natural contact infection. In this experiment 19 vaccinated fowls were exposed in an infected pen, along with 10 unvaccinated birds. The vaccinated fowls remained free while four of the unvaccinated birds contracted the disease. All vaccinated birds were given a test dose of 0.5 c.c. of  $1 \times 10$  dilution of virus after 15 days exposure in the pen, with the result that only one of the vaccinated fowls died of Ranikhet disease, whereas five of the surviving 6 unvaccinated fowls proved to be susceptible when used as virus reservoirs in later experiments on similar lines.

With regard to the infectivity of birds undergoing vaccination, 6 healthy fowls were placed individually in close contact with 6 vaccinated fowls. In no case did the healthy birds develop symptoms; further, mouth washings were taken from several vaccinated birds selected at random on the 4th, 5th, 6th and 7th day after vaccination and inoculated into healthy fowls. The disease was not transmitted.

We do not think it necessary at this preliminary stage to enter into a discussion of the findings reported. While the results obtained by the use of this form of vaccine are far superior to any other method tried in this laboratory, the limitations of the method at the moment are self-evident and further work will now be required to eliminate them or at least to define them precisely. It is hoped, however, that other laboratories having the necessary facilities will give the method a trial.

#### SUMMARY

The authors have described in detail the method of preparation of a vaccine for Ranikhet disease. The method consists in the adsorption of the virus from a spleen pulp suspension on an alumina gel. The mixture is then matured at a suitable temperature.

The results of preliminary experiments with this vaccine are reported and it is shown that the period for maturation is mainly controlled by the temperature at which this is carried out—the higher the temperature the quicker the maturation.

It has also been demonstrated that a fairly efficient vaccine may be obtained after maturation from 8-10 days at room temperature ( $14^{\circ}$ - $16^{\circ}$ C.), or at 1 to 4 days in the incubator ( $37^{\circ}$ C.). The shorter period of 24 hours in the incubator has given the best results, a very high proportion of birds receiving this vaccine develop a high degree of immunity. As far as can be judged by preliminary work, birds undergoing vaccination are non-infective, and, therefore, the use of this vaccine will not tend to spread the disease.



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## SELECTED ARTICLES

### COMMON DISEASES OF YOUNG CALVES AND THEIR CONTROL IN INDIA

BY

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(With Plates X to XIII)

THE cattle breeding and dairy industries annually suffer grave losses due to disease in young calves. Sufficient recognition has not hitherto been given in India to such losses, in which should be included not only fatalities among young calves which would have otherwise become valuable cows bulls or bullocks, but also the cost of looking after the diseased calves, inability to replace animals that are cast from the herd each year, expenses involved in purchasing fresh stock to keep up the strength of the herd and rearing of those calves which would in other countries have been disposed of as unprofitable. It is, therefore, of vital importance for the proper development of these industries in this country that losses on this account should be checked by keeping the diseases of young calves under control and to this end stock owners should take every opportunity to obtain the assistance of the Civil Veterinary Department.

At the Government Cattle Farm, Hissar, Punjab, where about 7,000 head of cattle are kept, including a dairy herd, these diseases have been observed to occur more frequently in the dairy calves than in those of the general herds. The diseases commonly encountered are white scour, navel-ill, pneumonia and ringworm. These as well as congenital blindness, rickets, etc., which are due to vitamin or mineral deficiency in the diet, are also common in Indian villages and certain dairy farms where calves are reared under less favourable conditions.

Calves are very liable to contract haemorrhagic septicaemia (*gulghotu* or *ghotwa*) and blackquarter (*phatsari*) through grazing over infected areas, particularly during the rainy season, and it is therefore advisable that they should be protected with vaccines against such diseases before they are sent out to paddocks.

The diseases of young calves may be conveniently divided into bacterial, nutritional and parasitic.

#### I.—BACTERIAL DISEASES

Bacterial diseases of young calves are mostly congenital, *i.e.*, infection occurs inside the uterus and the symptoms are manifested shortly after birth. In such cases the same organisms which cause trouble in the young can usually be isolated from the uterus of their dams. These infections may also take place after birth from external sources, and the earlier they appear in life the less are the chances of recovery.

The saying 'Prevention is better than cure' holds good in the control of congenital bacterial infections for which the following general measures of prevention are recommended, and they may advantageously be combined, in the event of actual outbreaks, with the curative measures to be described later under various diseases :—

1. Attention should be paid to the proper feeding and exercise of the herd and sanitation of the premises, as these factors have a great influence on the disease resistance of the herd and the production of healthy normal progeny.

2. The mating of parents with diseased sex organs should be avoided. Cows with an abnormal vaginal discharge or sires with infected genitals or temporarily sterile cows should not be used for breeding.

3. Cows should be dried six to eight weeks before they are likely to calve, in order to give them a chance to recover their health and vitality for the next parturition. They should be fed liberally on a well-balanced ration containing adequate proportions of minerals and vitamins. For the supply of the former, 3 to 4 ounces of bone-meal should be mixed daily with the concentrate ration to provide calcium and phosphorus. Small quantities of potassium iodide should also be added to provide iodine and rock salt to lick *ad libitum*. For the supply of vitamins green fodder should be provided throughout the year, if possible, and when required 2-4 ounces of cod-liver oil may be added daily to the concentrate ration just before feeding.

4. Cows should be prepared for calving by clipping hair from the hind quarters and legs and by daily washing and cleaning the external genitals, tail, and hind quarters with an antiseptic lotion (e.g. 1 in 1,000 solution of potassium permanganate) for a few days before calving. When about to calve the cow should be moved to a clean stall which has been thoroughly disinfected previously.

5. The calf should be received on fresh and unsoiled straw and its umbilical cord should be immediately ligatured with carbolized twine about half an inch from the abdominal wall, cut with a sterilized pair of scissors and the stump dressed with a tincture of iodine followed in a few minutes by an application of Stockholm tar.

6. Every calf should be allowed the colostrum of its dam for at least a few days. Colostrum is the most natural food for the calf, and is endowed with laxative property. It is also rich in minerals and contains certain substances (antibodies) which act as a preventive against various infections.

7. When calves are weaned they should be fed often, but with small quantities of milk at body heat. If they are fed with a large quantity of milk at one time it forms a big clot in the stomach which causes gastric irritation. If a calf is to be fed on separated milk instead of whole milk, this should not be done until the calf is four weeks old and the change should be brought about gradually. The cream that has been removed by the separator may be substituted by giving 1-2 ounces of cod-liver oil daily.

8. The dam's udder and feeding utensils should be kept clean in order to prevent infection taking place after birth through feeding. It is a good practice to scald the utensils thoroughly after cleaning.

9. Regular exercise out-of-doors is essential for calves in order to keep them fit and to prevent them from catching cold and pneumonia.

10. The calf pens should be built hygienically with concrete floors and be kept clean, properly drained and occasionally disinfected and white-washed.

11. If the calves are kept in small groups contagious diseases can be controlled more easily.

12. On infected farms the temperatures of all the new-born calves should be taken for at least 7 to 10 days from birth as in most of these congenital diseases the first indication is rise of temperature. Moreover, these diseases are more fatal to young calves, and the earlier these are detected the greater are the chances of their recovery with suitable treatment. Usually the normal calf is very active and vigorous and has a soft smooth coat. If it lies quietly or stands in a stupor or if the coat is rough, it may be taken that it is out-of-sorts and the source of the derangement should be ascertained.

13. The affected calves should be isolated immediately from the healthy and be provided with separate attendants. The premises and feeding utensils should be thoroughly disinfected. The healthy calves may be removed to fresh clean premises, preferably at a higher level.

14. The carcase of a calf that has died of any contagious disease should be either burnt or buried with lime six feet below the surface.

15. On the advice of a competent veterinary authority, calves born on farms infected with white scour or navel-ill may be given preventive inoculations against these diseases within a few days of the birth. Pregnant cows, especially those which habitually give birth to calves affected with these diseases, may also be similarly treated.

(i) *Calf septicaemia*.—In this disease the calf is born sick and weak, is unable to get up, shows high temperature and usually dies within twelve to twenty-four hours or a little longer after birth. On *post-mortem* examination the heart, serous and mucous membranes show haemorrhagic spots. The spleen may be slightly enlarged and congested. In such cases the dam usually has retained the placenta or shows symptoms of inflammation of the uterus and there may be a history of previous abortion or of the birth of a calf infected with navel-ill.

Very little can be done to save the calf affected with this disease. However, if possible, internal antiseptics and general stimulants such as a mixture of quinine sulphate (ten grains), sodium salicylate (thirty grains), potassium iodide (twenty grains) and aromatic spirits of ammonia (one drachm) in about two ounces of water may be given three or four times a day. A serum prepared against the organisms that are commonly encountered in such outbreaks may be used as a curative. General hygienic care and proper nursing may help the patient.

(ii) *White scour*.—In this disease the calf shows a rise of temperature before manifesting scour and there is dullness, depression and disinclination for food. A few hours or a day later the faeces become thin and are of a yellowish or white colour with a foetid odour. The affected calf has a staring coat and cold limbs, is very weak and is unable to stand. When young calves get white scour, older calves may subsequently contract the infection from them and an outbreak may result, but the younger calves are affected more seriously and the losses will be much greater than in the older calves. When the disease develops within a few hours of birth the calf may die within two days. In

some cases there is a tendency for white scour gradually to merge into calf pneumonia.

If diarrhoea has not yet commenced and there is only a rise of temperature, isolate the affected calf, reduce the food and immediately inject subcutaneously 15 c.c. of the white scour serum available for the purpose, the injections being given every three hours for the first day. If the calf is very weak and shows lassitude, a pint of warm water containing one tea-spoonful of sodium bicarbonate and a drachm of aromatic spirit of ammonia may be given morning and evening an hour before feed. This will act as a stimulant and will counteract the over-acidity which is common in such cases. Salol or sodium salicylate or bismuth subnitrate in half to one drachm dose may be given three times a day with good results.

When diarrhoea occurs, discontinue the use of milk as an article of diet at once and substitute barley water at body heat. Sodium bicarbonate may be added. When barley water is not available, lime water to which a little wheat flour has been added may be used instead and it may be drenched, if necessary. The calf should be blanketed and kept in a clean warm place. The extremities may be hand-rubbed and bandaged. About 30 c.c. of the white scour serum may be given intravenously.

(iii) *Calf pneumonia*.—In this disease, which may accompany or follow white scour, there is fever, nasal discharge, hacking cough and laboured breathing (Plate X, fig. 1). The calf may be unable to rise and may develop dropsy of the dependent parts. Usually both lungs are found to be affected and on *post-mortem* examination they may present a marbled appearance with thickened interlobular septa. In older calves the condition may become chronic with abscess formation in the lung (Plate X, fig. 2).

In this disease the calf should be blanketed and kept in a clean, warm place and its extremities hand-rubbed and bandaged. Preventive inoculations with haemorrhagic septicaemia serum is indicated in such cases, because generally the organism of this disease is prevalent in those herds where pneumonic symptoms predominate. Good results are likely to be obtained from the application of a mild mustard plaster or a stimulating liniment to the chest wall and from the use of medicated inhalations, e.g. carbolic acid or eucalyptus, and general stimulants and internal antiseptics such as those recommended under calf septicaemia.

(iv) *Navel-ill*.—In this disease, which is mostly congenital, the navel may be somewhat swollen with a foul-smelling discharge (Plate X, fig. 3). There is fever, lassitude and weakness and the calf may remain lying down. Infection from the navel may extend to the liver and also to the various joints and these may form suitable sites for the propagation of bacteria. The affected joints become swollen, hot, tense and painful and there is marked lameness.

When the joints have become affected, curative treatment is far from satisfactory. When synovial sheaths alone are affected, it is comparatively easy to treat. In the first instance the umbilicus should be carefully examined. If there is an abscess it should be opened in a place where there are no other animals and the evacuated pus should be carefully disposed of in order to prevent the spread of infection. The abscess cavity should then be irrigated with a disinfectant lotion, followed by a dressing consisting of equal parts of



carbolic acid and glycerine, and subsequently treated daily with milder dressings such as 2½ per cent carbolic lotion or tincture of iodine till the wound is healed. When there is an abscess in the region of a joint, it should be opened, evacuated, irrigated with a disinfectant lotion followed by an injection of clove oil into the wound which should then be bandaged. If the abscess involves the joint proper and it has reached the stage of purulent arthritis, treatment is rarely worth while. In the case of non-suppurating inflammation of the joint, a weak biniodide of mercury blister or a mixture of liniment camphor co. (one part), liniment belladonna (two parts) and liniment saponis (three parts) may be applied locally. Stimulants and internal antiseptics may be administered, e.g. 1 to 2 drachms of aromatic spirits of ammonia and ½ to 1 drachm of salol in about two ounces of water, thrice daily. Calcium sulphide in 15 grains dose mixed with simple syrup may be given thrice daily as it has been found to be good in all pyaemic conditions.

(v) *Calf diphtheria*.—This is another contagious bacterial disease of young calves but the infection in this case is acquired after birth. It is characterized in its course by diphtheritic inflammation of the mucous membrane of the mouth, from where it may extend further. Generally the disease is confined to such farms or premises where calves are reared under insanitary conditions. It is quite distinct from human diphtheria and is usually conveyed through unclean milk pails and feeding utensils. The organism of the disease remains alive for a considerable period in sheds and is therefore easily transmitted to healthy calves. Poorly fed calves are most susceptible and the disease is more common among pail-fed calves during early spring and late autumn months when the weather is changing and is rather cold. Infection is facilitated by the eruption of teeth which causes injuries to the mucous membranes of the mouth.

In an outbreak, the majority of calves become affected, weak and poorly nourished calves being usually the first to be attacked. The affected calves show a rise of temperature, unthriftiness, impaired appetite, salivation, coughing, dirty-yellow nasal discharge and red granulating ulcers and yellow patches on the mucous membrane of the lips, gums and inside the mouth. These mouth lesions bleed easily and make it difficult for the affected calf to pick up solid food. The infection may spread to the intestine and lungs. The bowels may become irregular, with a tendency to diarrhoea, and in some cases pneumonia develops and increases the mortality. Severe cases die in three to five days whereas mild cases may recover with suitable treatment in about three to four weeks but the animals' growth remains stunted. On *post-mortem* examination the yellow patches and ulcers referred to above may be found to have extended to the pharynx, nasal cavity, larynx, lungs and liver and at other times in the intestine (Plate X, fig. 4).

For the control of this disease milk pails, feeding utensils or troughs should be examined in order to determine the source of infection. One should be particularly suspicious about those that are made of wood. All such utensils or troughs should be thoroughly scalded. The calves should be examined and those affected should be isolated and kept in a clean warm place. The premises should be thoroughly disinfected. The mouth lesions should be cleaned with 1 in 1000 solution of potassium permanganate, and the yellow patches may be

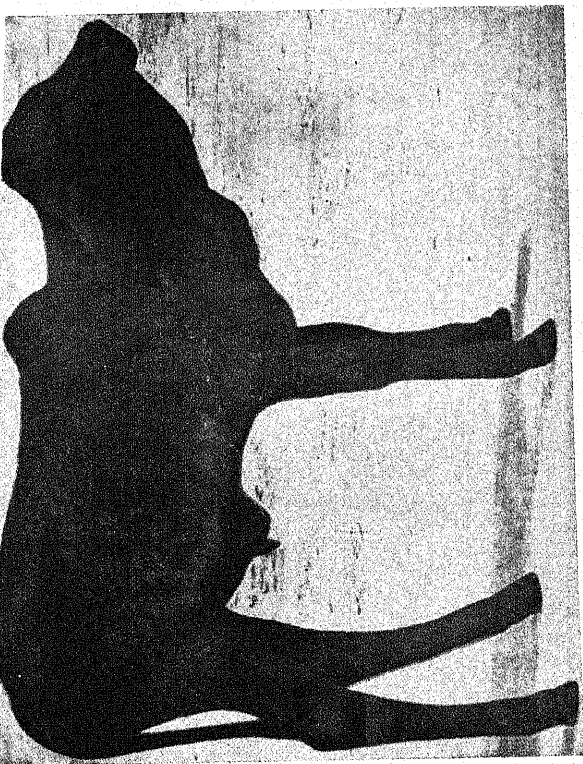


FIG. 1. A typical case of calf pneumonia [After J. F. Shirlaw]



FIG. 3. A hill calf affected with navel-ill

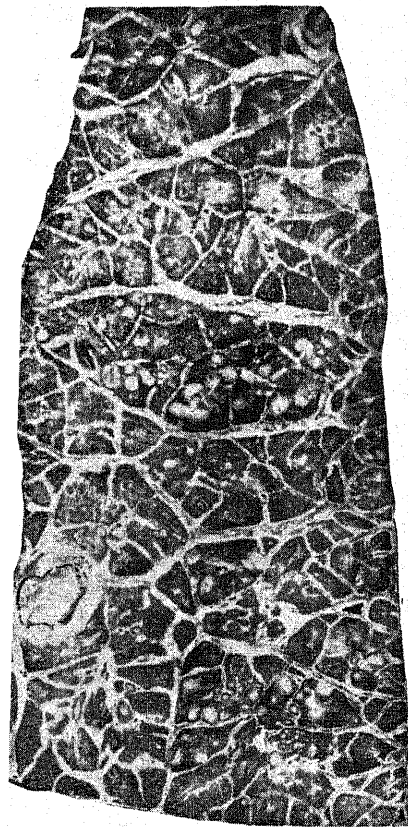


FIG. 2. The cut surface of a lung showing typical lesions of calf pneumonia [After J. F. Shirlaw]

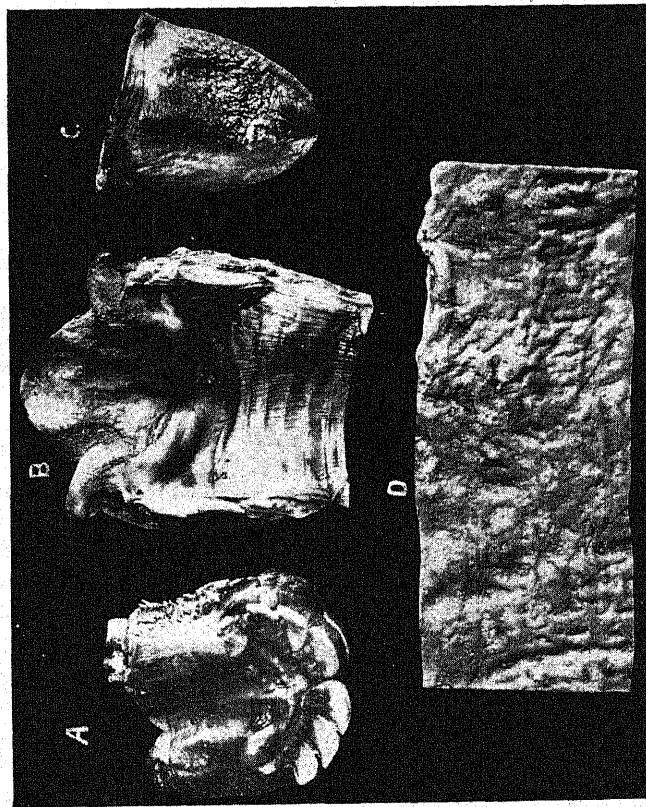
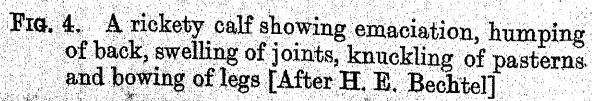
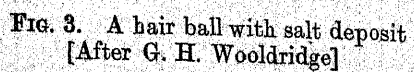
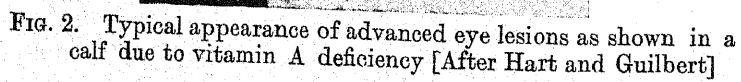
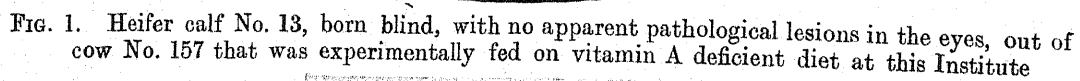


FIG. 4. Diphtheritic ulceration on the (A) gums and lip, (B) larynx, (C) tongue and (D) intestine





curetted. Tincture of iodine may then be painted over the lesions. Before feeding, the milk should be warmed and about half a drachm of potassium chlorate for each calf should be dissolved in it. Internal antiseptics and stimulants, e.g. salol ( $\frac{1}{2}$  drachm), sodium salicylate ( $\frac{1}{2}$  drachm), and aromatic spirits of ammonia (1 drachm) in about two ounces of water may be given to the calves two or three times a day. In order to keep up the vitality of the patient, artificial feeding with whole milk and eggs may have to be resorted to in some cases.

## II.—NUTRITIONAL DISEASES

In new-born calves there occur a number of diseases which are the result of feeding cows on deficient and unbalanced diet, especially during pregnancy. Of these, mention may be made of congenital blindness, pica and rickets. Nutritional deficiency in the cow is reflected in the calf during its foetal development and while on milk. Such a deficiency in the dam may also result in abortion, or the birth of a dead calf, of a weak and undersized calf that may die soon after birth. Even those calves that are born normally to such dams, and are apparently healthy, possess a greatly lowered resistance to disease. All these pathological conditions are very difficult to cure but they can be easily prevented by feeding cows on diets containing suitable proportions of vitamins and minerals.

Although goitre, which is prevalent in other species of animals, is not so far known to affect calves in India, nevertheless it is liable to occur chiefly in places that are away from the sea at high altitudes and around large lakes where the soil and water are deficient in iodine and its occurrence in such areas can be easily prevented by using iodised salt licks or by occasional administration of potassium iodide in small quantities in their diet.

(i) *Congenital blindness*.—It would appear that the condition is especially widespread in the Indus valley, certain parts of the Punjab and Delhi where green fodder is not included in the diet of cattle for considerable period during the year. It is also of interest that night blindness, which is reported to disappear on feeding fresh green fodder, is prevalent in these areas. The disease becomes much more serious in those herds where intensive methods of breeding and milk production are practised because under these conditions any nutritional defect brought about by lack of vitamins or minerals in the diet becomes more pronounced. In such herds, the incidence of congenital blindness may be as high as 30 per cent or more. It has been observed that cows which have had intermittent periods of sterility do not produce blind calves. Such sterility prevents the depletion of the mineral and vitamin reserves of their bodies by frequent pregnancies and thus enables them to deliver normal calves occasionally.

The calves may be born blind of one or both eyes or the sight may be only impaired at birth and complete blindness may follow gradually. In most cases the blind calves do not show any apparent pathological condition of the eyes, and these outwardly appear to be perfectly normal (Plate XI, fig. 1). In such cases degenerative changes are usually found in the optic nerves. However, in some cases the cornea and other structures of the eye may show

inflammatory changes followed by an opacity in the eye (Plate XI, fig. 2). Sometimes these calves also develop convulsive fits which may be associated with vitamin A deficiency.

The occurrence of congenital blindness in calves can be successfully prevented by including green fodder or two or four ounces of cod-liver oil in the daily ration of cows especially during pregnancy.

(ii) *Pica*.—A large quantity of calcium and phosphorus is required for growth of the foetus inside the uterus and a similar quantity is also passed out from the cow's body in the milk. She, therefore, requires plenty of these minerals in her food during pregnancy and the lactation period in order to cope with this demand and to make up any losses in the bones which act as body reserves for these minerals. If the demand on these body reserves is too large and the losses are not made good, the cow as well as her milk yield suffers, and the calves may be born weak or dead. Such cows may even fail to breed or may abort. Calves require plenty of calcium and phosphorus for bone development and it is, therefore, essential to provide them with a sufficient quantity of these minerals in the diet. If by any means calves suffer from a lack of phosphorus they generally develop pica. It may also be caused by an insufficiency of sodium salts.

Affected calves have a tendency to lick and to gnaw almost any foreign object which they come across, e.g. wood, earth, soiled litter and even excreta. Their appetite for normal food is very capricious. They become uneasy and depressed and lose condition. They show intermittent tympany and irregular bowels. If left untreated they become thin and wasted and die from malnutrition and exhaustion after a period of suffering which may last for several months. They lick one another and by so doing considerable quantity of hair may accumulate in the rumen, the constant movement of which may convert it into a ball or a short cylinder. Sometimes salts may deposit on a hair ball giving it a highly polished surface (Plate XI, fig. 3). On account of the changing position of these balls intermittent flatulence or impaction may result.

The best method of preventing this disease is to supply plenty of calcium, phosphorus and sodium in the diet of the cows during pregnancy and to the calves after birth. Calves that are fed on liberal amounts of milk will receive enough calcium and phosphorus from this food. To compensate any deficiency of these minerals the best method is to leave a mineral mixture consisting of 200 lbs. of bone-meal, for the supply of calcium and phosphorus, and 150 lb. of common salt, for the supply of sodium, contained in a box protected from rain in the calf-pens and cow-sheds so that they may eat it as they need. The digestive disturbances may be treated by giving castor oil (2-4 ounces) followed by digestive tonics like gentian (1-2 drachms), ginger (1-2 drachms) and nux vomica (10-20 grains). When a hair ball is suspected, treatment is not satisfactory as the condition is likely to prove fatal sooner or later.

(iii) *Rickets*.—This is a disease of young animals caused by faulty nutrition and is characterized by constitutional debility, together with enlargement of the ends of the long bones and a diminution in their resistance. There is a deficiency of lime salts and an excess of organic matter in the affected bones. The quantity of lime salts, which form the framework of the bone, limits the



organic matter in it. Absence or deficiency of vitamin D (antirachitic) which controls the metabolism of calcium and phosphorus, deficiency of lime (calcium) and unsuitable calcium-phosphorus ratio in the diet are the chief aetiological factors, although under the same feeding conditions some may suffer from this disease while others may escape due to certain individual differences.

The affected animal shows stiffness of the limbs, disinclination to move and a tendency to stand with the back humped. The long bones in the limbs become supple and curved under the weight of the body. Their ends which form the joints become swollen. This swelling is more commonly seen at the hock and knee joints (Plate XI, fig. 4). The ends of the ribs also become enlarged and may be easily palpated or seen. The affected animal loses appetite for its normal food but licks the walls and earth, probably in order to get lime salts in which its body is deficient. The disease runs a chronic course and if an animal is left untreated it dies of exhaustion or of congestion of the lungs due to its constantly lying down on one side.

The method of its control consists in providing plenty of well-balanced diet, reasonable exercise in the open and hygienic care for the pregnant cows and for the new-born calves. Sunshine is very essential in the prevention of this disease because it converts ergosterol, a precursor of vitamin D which is present in the skin, into vitamin D. Whole milk cannot be relied upon to supply enough vitamin D for the needs of the calf and skimmed milk is certainly deficient. Of the natural foods sun-cured hay is only reliable source of vitamin D. The calf which is given this hay liberally will not ordinarily have rickets. If at any time symptoms of rickets develop, irradiated yeast or irradiated ergosterol or purified cod-liver oil may be given in order to supply vitamin D along with bone-meal which would supply both calcium and phosphorus in a suitable proportion and plenty of nutritious diet. Light exercise, if possible, may be given in the open where there is plenty of sunshine. The affected limbs may be supported by means of splints but one should not attempt to reduce the swelling of joints by applying pressure bandages which may result in sloughing. With suitable treatment recovery may occur in a considerable number of cases, provided they are in the early stages of the disease. A deformity in the affected limbs or joints may remain even after the treatment which is useless in advanced cases. On the whole, prevention is more satisfactory than the treatment of affected individuals.

### III.—PARASITIC DISEASES

#### A. *Helminthic*

Of the helminthic diseases, parasitic bronchopneumonia, parasitic gastritis, intestinal infestation with the large round worm (*Ascaris vitulorum*) and tape worm (*Moniezia expansa*) and infestation with the eye-worm (*Thelazia rhodesi*) are of common occurrence in calves. The control of these diseases may be largely effected by adopting the following general measures recommended for their prevention rather than waiting to treat the clinical cases when

they occur, because by the time they attract attention the parasitic infestation in a herd may have become heavy and have reached a serious stage :—

1. Damp and low-lying places provide an ideal place for the development of worm larvae and the breeding of intermediate hosts of certain worms. Such places should, therefore, be avoided or drained. Ditches which cannot be drained properly may be filled in with earth.

2. Keep the young stock which are more susceptible to parasitic infestations away from the older animals which are often carriers of parasites and from the infected grazing areas used by them.

3. In order to avoid over-stocking, which is liable to increase parasitic infestation, keep calves in small batches, according to age, in separate pens.

4. Paddocks for calves should be used in rotation. The ground should be ploughed and used for cultivation when not required as a paddock. This procedure will kill the worm eggs and larvae.

5. Frequent removal and proper disposal of the excreta is necessary. It may be stored in a pit and used as manure in fields that are under cultivation.

6. Watering and feeding troughs should be kept clean and be at a higher level than the ground in order to avoid contamination.

7. Build up the resistance of the herd and that of the new progeny by feeding the cows on nutritious and well-balanced diet, paying special attention to its vitamin and mineral contents.

8. Obviously affected animals should be isolated from the rest and the premises should be thoroughly cleaned and disinfected. Animals in both lots should be given a suitable treatment with vermicides.

9. Regular drenching of the stock with vermicides, as recommended later, helps a great deal in keeping the parasitic infestations on a farm under control.

(i) *Parasitic bronchopneumonia*.—This is caused by a thread-like round worm, known as *Dictyocaulus viviparus*, which is of the thickness of twine and is about 1½ to 5 inches in length (Plate XII, fig. 1). The parasite inhabits the trachea, bronchi and lungs whence the worm eggs are coughed up and swallowed. In the intestine the eggs hatch and young larvae are passed out in the faeces. Under favourable conditions of moisture and temperature these larvae survive and are swallowed by other animals along with the grass and reach the lungs *via* the blood stream.

The common symptoms are nasal discharge, paroxysms of cough with expulsion of mucus which is sometimes mixed with blood and always contains worms and their eggs. The coughed-up mucus may be swallowed and passed out with the faeces, thereby spreading the infection on the pastures and farm premises. The animal gradually loses condition and becomes anaemic and its respiration becomes accelerated. Swellings may appear on the dependent parts of the body. The affected lung shows patches of consolidation with worms and their eggs in the air passages. The condition can be diagnosed by observing the symptoms and examining the parasite and the eggs, under the microscope, in the coughed-up mucus and nasal discharge (Plate XII, fig. 2).

For the control of this parasitic infestation, the affected animals should be isolated and the infected premises vacated. Low-lying places and ditches should be avoided. The animals should be fed liberally on nourishing diet

and provided with clean drinking water and should not be overcrowded. Regular drenching of the whole stock with a suitable vermicide mixture, as recommended later under the parasitic infestations of the digestive tract, is likely to prove useful. Those vermicides which are excreted by the lungs and act as pulmonary and bronchial disinfectants are especially useful, e.g. turpentine oil (1-3 drachms) or carbolic acid (5-15 minims) or lysol (5-15 minims) given in milk. This method has the additional advantage of destroying stomach parasites which are so often simultaneously present. Generally the treatment of individual cases is difficult on account of the peculiar location of these worms. However, they may be killed by pouring about  $\frac{1}{2}$  to 1 drachm of chloroform into the nostrils or better 3 to 4 drachms of the following iodine mixture may be given slowly by means of an intratracheal injection, to be given by a qualified veterinarian :—

Iodine 1 part.  
Potassium iodide 10 parts  
Distilled water 100 ..

(Mix up and make into emulsion by adding equal parts of olive oil and turpentine oil.)

The general measures recommended for the prevention of parasitic diseases may be adopted.

(ii) *Parasitic gastritis*.—This is caused by two species of wire worms (*Haemonchus contortus* and *Mecistocirrus digitatus*). The former parasite (Plate XII, fig. 3) is about an inch long and its female possesses a peculiar appendage covering the vulva situated at about the posterior fourth of the body and visible to the naked eye. The latter parasite (Plate XII, fig. 4) which is more common in India is about 1 to 1½ inches long, is stouter than the former parasite and its females do not possess any appendage over the vulva. The males of both worms are comparatively small and possess a peculiar expansion or 'bursa' situated at the posterior end and visible to the naked eye. In either case the eggs (Plate XII, figs. 6 and 7) are passed out in the faeces and hatch under suitable conditions of warmth and moisture, and the young larvae crawl up the grass blades and infest any animal ingesting them.

The parasites cause marked digestive disturbances, loss of appetite and constipation followed by diarrhoea. There is progressive loss of condition (Plate XII, fig. 5), the animal becomes anaemic and in advanced cases swellings develop on dependent parts of the body. The animal may show convulsions and die of extreme emaciation. The condition can be diagnosed by the symptoms mentioned above, detection of the worm eggs in the faeces and parasites in the fourth stomach on *post-mortem* examination.

On an infected farm parasitic gastritis can be controlled by regular drenching of young stock with 3 to 4 ounces of 1 per cent solution of copper sulphate. It is not necessary to fast the animals before drenching. After drenching, the animals should not be given any feed or water for 2 to 3 hours. This treatment does not require subsequent purgation. The solution is best prepared by dissolving 4 ounces of copper sulphate crystals in a pint of boiling water in an enamelled or earthen vessel and then diluting it to 3 gallons with cold water.

(iii) *Intestinal infestation with large round worms*.—This infestation is due to *Ascaris vitulorum*, the male of which possesses a specially curved tail and is

smaller and thinner than the female, measuring 6 to 10 inches in length and  $1/6$  to  $1/5$  inch in thickness. The female (Plate XII, fig. 8) has a straight tail and measures 8 to 12 inches in thickness. The worm eggs (Plate XII, fig. 9) are passed out in the faeces and under suitable conditions of temperature and humidity embryos appear in them in about three weeks, after which they are infective. On ingestion with contaminated food or drinking water the larval worms are liberated in the digestive canal, thence enter the blood vessels and reach the various organs, e.g. heart, lungs and liver. In about two weeks from the time that they first enter the host they reach the digestive canal a second time.

These parasites do not affect the health of the animal unless they are numerous. Heavy infestation may result in chronic inflammation of the intestine, and continued diarrhoea or diarrhoea and constipation may alternate. The affected animals lose condition, appear dull and their coats become dry and harsh. Their development is arrested and they become pot-bellied. Occasionally the parasites may cause intestinal obstruction. The condition can be diagnosed by the clinical symptoms, detection of the worm eggs in the faeces and the parasite in the intestine on *post-mortem* examination.

The treatment of affected animals with ordinary vermicides, e.g. turpentine oil (one ounce in about half a pint of linseed oil) gives quite satisfactory results. This treatment should be followed in about 12 hours by a dose of about 4 ounces of castor oil.

(iv) *Intestinal infestation with tape worms.*—The common tape worm which infests the intestine of calves in India is *Moniezia expansa* (Plate XII, fig. 11) measuring 3 to 20 feet in length and about half an inch in breadth. The tape worm possesses a globular head, a narrow neck and a segmented body in which each segment is a sexually complete individual possessing both male and female organs. The terminal segments containing large number of eggs constantly break off and are passed out with the faeces. Probably under suitable conditions these eggs undergo further development for about two months and become infective. Calves may get infected soon after birth and the worm attains the adult stage inside the intestine in about six weeks' time.

When the parasites are present in small numbers no marked symptoms may be observed but when they are in large numbers the animal appears dull, the visible mucous membranes are pale and digestive disturbances set in. Rumination becomes irregular. At first there is constipation but it is soon followed by diarrhoea and mature segments of the parasites containing the eggs are passed out in the faeces. There are indications of colic and the worst-affected animals will follow the herd with difficulty. There is progressive weakness and exhaustion and the affected calves remain stunted in their growth. The condition can be definitely diagnosed by the detection of the worm segments or eggs (Plate XII, fig. 10) in the faeces and the entire parasites in the intestine on *post-mortem* examination.

A drug should be considered to have produced the desired effect only when the heads of the parasites are passed out in the faeces and one should look for these after a vermicide drench has been given. One per cent solution of copper sulphate, as recommended in parasitic gastritis, also proves effective against





FIG. 11. *Moniezia expansa* (adult). Note the head, neck and segmented body



FIG. 8. *Ascaris vitulorum* (female)

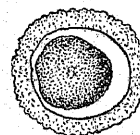


FIG. 9. An egg of *Ascaris vitulorum*

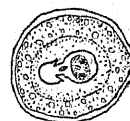


FIG. 10. An egg of *Moniezia expansa*

FIG. 2. An embryonated egg of *Dictyocaulus viviparus*.

FIG. 1. *Dictyocaulus viviparus* (A) Female, (B) Male

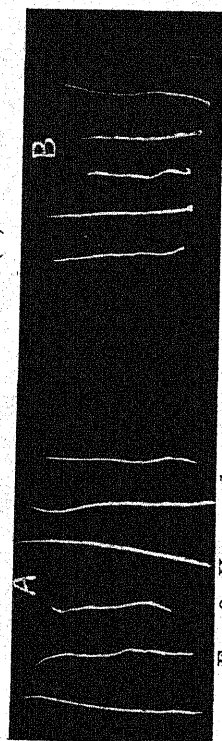


FIG. 3. *Haemonchus contortus* (A) Females, (B) Males

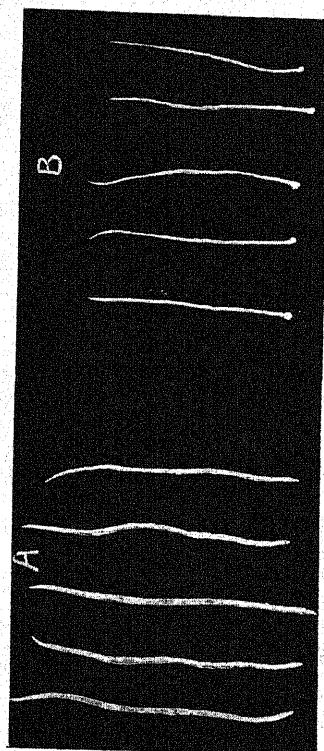


FIG. 4. *Mecistocirrus digitatus* (A) Females, (B) Males



FIG. 5. The condition produced by a heavy parasitic infestation of the digestive tract in a calf [After D. W. Baker]

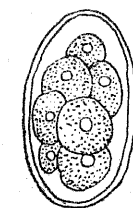


FIG. 7. An egg of *Mecistocirrus digitatus*

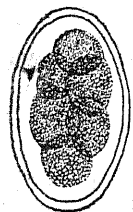


FIG. 6. An egg of *Haemonchus contortus*



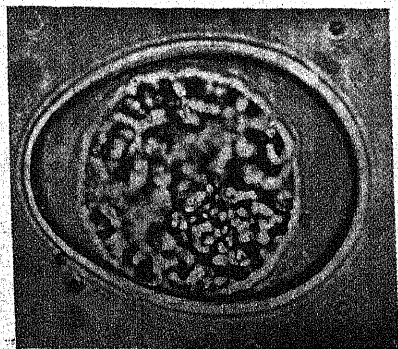


FIG. 1. *Eimeria zurni* ( $\times 2,000$ )  
[After C. M. Wenyon]



FIG. 2. A calf affected with sarcoptic mange. Note the thickened, wrinkled and scaly condition of the skin  
[After Huttyra & Marek]



FIG. 4. A calf affected with ringworm. Note the circular lesions

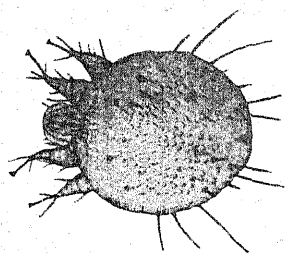


FIG. 3. *Sarcoptes scabiei*

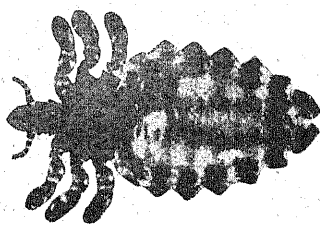


FIG. 7. *Haematopinus eurysternus*  
[After M. Innes]



FIG. 8. *Linognathus vitalis*  
[After M. Innes]

FIG. 5. *Boophilus australis*

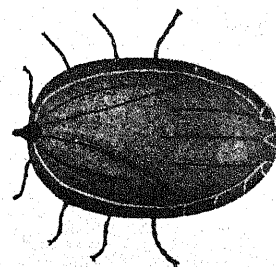


FIG. 6. *Hyalomma aegyptium*



FIG. 9. *Trichodectes scalaris*  
[After M. Innes]

tape worms provided one per cent by weight of tobacco dust is added. The tobacco dust should be steeped overnight in a little cold copper sulphate solution and then added to the rest of the solution. It is not necessary to follow up with a purgative. Another method of treatment is to give two doses of kamala (1 to  $1\frac{1}{2}$  drachms) in about 4 ounces of castor oil the same day. Freshly ground arecanut (two drachms) may be given in milk twice daily for a week and be followed by a purgative, e.g. magnesium sulphate (6 ounces) dissolved in water.

(v) *Eye-worm infestation*.—This infestation is caused by a thread-like round worm about  $\frac{1}{2}$  to 1 inch long, called *Thelazia rhodesi*. This is usually found in the conjunctival sac but it may invade the anterior chamber of the eye, when the condition becomes much more serious. A large number of calves may suffer from this eye trouble on a farm. When the worms are few the symptoms may be so slight that the condition may remain undetected. In some cases, however, conjunctivitis, lachrymation, and fear of light may be present. When the worms are present in large numbers these symptoms are more intensified and ulceration and opacity of the cornea may result rendering the animal blind. The condition can be diagnosed by the symptoms described above and by the detection of the parasite in the affected eye.

Treatment consists in the removal of the worms by means of soft camel-hair brush or a fine pair of forceps, using a local anaesthetic if necessary, and then treating the inflammatory condition of the eye by means of antiseptic eye lotions, e.g. silver nitrate (1 to 2 grains) in an ounce of distilled water.

#### B. Other parasitic diseases

(i) *Coccidiosis*.—It is essentially an enzootic disease caused by a double-contoured oval parasite called *Eimeria zurni* (Plate XIII, fig. 1) inhabiting the intestine and setting up severe inflammation of the intestine. The disease is more common during the wet season in low-lying and marshy areas. The infection takes place by means of ingestion of contaminated food and water.

The disease appears in an acute form in calves, but older animals show more resistance which is liable to be broken down by devitalising diseases, especially those which involve the digestive tract, e.g. rinderpest. The affected animal stands with arched back and head held forward and shows loss of appetite and severe watery diarrhoea accompanied by the passage of blood and mucus, and the rectum may prolapse on account of too much straining. Anaemia and emaciation may be present and there may also be febrile symptoms and the animal may succumb. The older affected animals, though they may not show any symptoms of the disease, contaminate the pastures and byres and thus spread the infection to young susceptible animals. On *post-mortem* examination the intestinal mucous membrane, particularly of the large intestine, appears congested, inflamed, thickened and covered with flakes of clotted blood. In severe cases the epithelium of the intestinal mucous membrane may be denuded in certain places giving it a roughened appearance. The mesenteric glands may be congested and enlarged. The diagnosis of this condition can be readily made by the detection of the coccidium in the faeces with the aid of a low-power microscope.

The disease may be controlled by examining all the cattle on a farm and separating the affected animals from the non-affected ones. They should be removed from the pasture where they developed the disease and kept on high grounds or in clean dry sanitary byres until they are satisfactorily treated. Young cattle should be separated both in the byres and at pasture from the adult cattle which may be the carriers of the infection. The byres should be kept clean and the litter should be removed frequently and burnt. The animals should be prevented from drinking stagnant polluted water. Medicinal treatment in some cases may prove useful. One to two table-spoonfuls of the mixture containing two parts of ferrous sulphate, two parts of sulphur, and six parts of common salt may be given in the grain feed. Enema with luke-warm one per cent solution of alum and tannic acid is also recommended.

During the course of the treatment the animal should be kept comfortable and well protected from the weather and be fed on dry nutritious diet.

(ii) *Mange*.—The term mange or scabies is applied to a class of contagious skin diseases caused by mites, manifested by itching and eczema of the skin and resulting in the loss of hair and scab formation. There are three different mites which produce mange in cattle, namely *Sarcoptes scabiei* (Plate XIII, fig. 3) which causes the so-called sarcoptic mange and particularly attacks areas around the eyes, cheeks and neck, *Psoroptes communis* which causes the so-called psoroptic mange and attacks the sides of the neck, shoulders, base of the horns, root of the tail and back, and *Symbiotes (Chorioptes) bovis* which causes a type of mange noticeable at the base of the tail but which may extend towards the anus and inside the thighs. The lesions produced in all the three types and their treatment are very similar. Sarcoptic mange is the worst type of mange as its parasite burrows under the surface of the skin and remains embedded, especially during winter, and is therefore not easily affected by medicinal dressings. The disease spreads by direct contact or through contaminated yards and houses, grooming utensils, clothing, etc. After infection it may take about a month for noticeable symptoms to develop. It develops more readily in unthrifty animals during the winter months. The parasites are specific for cattle and do not thrive on other species of animals and man.

The first noticeable symptom in mange is constant rubbing and scratching of the skin. The skin is first covered with small pimples and scab, and after the hairs are rubbed off, bare patches appear on the affected parts. Later the skin becomes thickened, wrinkled into folds and cracked. There is marked loss of condition and even emaciation. During the warm weather, when pastures are also luxuriant, the condition may temporarily improve and remain undetected. All the three types of mange may be readily diagnosed by the detection of parasites in scrapings from the lesions, when these are examined on a dark background with a hand lens or under the low power of a microscope.

All the affected animals should be strictly isolated till cured and the infected premises, utensils and clothing should be thoroughly disinfected. The bedding and litter of the infected animals should be burnt. In the treatment of individual cases the hair should be clipped, if the affected area is not too large and the weather is warm. The clipped hair should be completely burnt.

Wash the surface with soap and warm water, clean it, allow it to dry and then apply the following dressing by means of a suitable brush :—

Sulphur 2 parts  
Oil of tar 1 part  
Potassium bicarb 1 part  
Raw linseed oil 8 parts

(Gradually heat the ingredients together and stir till thoroughly mixed.)

This dressing may be applied warm at a temperature that is slightly higher than the body temperature (105°-110°F.), so that its consistency remains thin and it may be applied more easily. The application should be left on for about 10 days and the surface of the body should then be washed and a second application given as before. Ordinarily two applications are sufficient to effect a cure but in obstinate cases another application may be given. When the number of animals to be treated is large they may be dipped in the following dipping solution, preferably at 105°-110°F. :—

Sulphur 24 lb.  
Unslaked lime (ordinary) 10 lb.  
Water (preferably soft) 100 gallons

In preparing this mixture, the lime should be slaked to form a thick paste, the sulphur should then be added to it and thoroughly mixed. To this, 25 to 30 gallons of boiling water should be added and the mixture should be boiled and stirred for two hours. Decant the fluid and add water to make 100 gallons. The capacity of a dipping vat can be easily determined by the following formula :—

Average length in inches × average width in inches × depth in inches

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=the capacity of the vat in gallons.

The animals should not be thirsty or hungry or overfed before dipping. They may be rested, if they have travelled a long distance, prior to dipping. The animals' bodies should be free from injuries and there should not be any projecting nails or similar objects in the vat which may injure the animal's body. The dipping solution may be stirred by means of a bucket or a plunger before the dipping commences. The animals should be completely dipped for 2-3 minutes at least, ducking their heads once or twice, and dipping should be repeated in 10-15 days. Immediately after the dipping the animals should be protected from exposure to cold. Dipping may be avoided during very cold weather. All the infected and exposed animals should be dipped. Following each dipping the yards and sheds occupied by them should be cleaned and disinfected and the animals then put in clean yards. The animals should be looked after well and fed nicely.

(iii) *Ringworm*.—This is a contagious skin disease caused by a parasitic fungus, known as *Trichophyton tonsurans*, and characterised by the formation of circular patches covered with scales, scabs and short and uneven hair stumps. It occurs most frequently among calves, especially during the winter months, and when they are kept undernourished and overcrowded in insanitary buildings. The vitality of the parasite is very great and it may live in a dormant state for several months in damp stables and may be carried from year to year, resulting in annual outbreaks of the disease. It spreads from one



animal to another by direct contact and indirectly by posts and other fittings in the calf pens, against which the infected calves may have rubbed their bodies or which may be contaminated by clothing or grooming utensils used on infected calves.

In calves the lesions are commonly found round the eyes, base of the ears or neck, shoulders, mouth and lips (Plate XIII, fig. 4). The parasite attacks the skin and destroys the hair which becomes brittle and breaks off, so that the disease is manifested by circular bare patches of skin which soon get covered with bran-like scales, but after a time the patches become covered with scabs of silvery grey colour. Sometimes these scabs crack and bleed, especially when the animal rubs the lesions against hard objects which it does owing to the irritation. The lesions do not heal spontaneously but they clean up sufficiently during summer and readily respond to proper treatment.

In order to control an outbreak of this disease the affected animals should be separated from the non-affected ones, the premises should be kept clean, dry, properly ventilated and disinfected, and the litter should be removed daily and burnt. Clothing, grooming utensils, etc. should be disinfected by boiling or soaking in 10 per cent carbolic solution or 1 in 1000 solution of mercury perchloride. Disinfection of walls, floors, partitions and other fittings should also be done carefully. The calves should be brushed, groomed and kept clean. A liberal diet should be allowed. Cats and dogs should not be allowed to run about the infected premises as they may disseminate the infection. The affected calves should be looked after by separate attendants. The best way of treating the lesions is to remove hair from round about them, soften the scabs with warm water and soap and remove all the debris, which should be burnt. Allow the parts to dry and then apply tincture of iodine or equal parts of tincture of iodine and vaseline or 1 in 40 ointment of biniodide of mercury. For widespread lesions one part of sulphur, one part of potassium carbonate, one part of oil of tar with 8 parts of lard or oil is quite good. A case should be considered as cured only when there is no longer a scabby condition of the skin and a good smooth crop of new hair grows.

(iv) *Tick infestation*.—There are a variety of ticks found infesting cattle in India and *Hyalomma aegyptium* (Plate XIII, fig. 6) and *Boophilus australis* (Plate XIII, fig. 5) are the more common ones. The latter tick transmits bovine piroplasmosis which is commonly known as red-water in cattle. Since calves possess a considerable degree of resistance to piroplasmosis it does not appear in them in its clinical form in spite of their harbouring the infected tick. They may, however, develop a mild attack and become immune to this disease for the rest of their lives. The harm, therefore, done by the ticks to calves is due only to their sucking the blood of the host, which becomes anaemic and to their inoculating into the host's body a poisonous saliva which creates uneasiness and emaciation so that the affected young animal develops poorly. The skin may appear rough and uneven on account of the attachment of ticks, which are found more numerous on the neck, ears, navel, thighs, etc. Pustules and ulcers may develop when the ticks have been torn from their host by licking or rubbing.

The best method of eradicating ticks from a herd is to burn the vegetation from the pastures, cultivate or change the pastures and house the animals in pukka buildings where there are no cracks in which the female tick can



deposit her eggs. The building should be kept clean and disinfected and the animals should be dipped occasionally in a suitable tick-killing solution, or this may be applied to them with a brush or cloth. When the number of animals to be treated is small a suitable tick-killing solution, as recommended below for dipping, may be applied by means of brush or cloth, or a spray pump may be used for the purpose with satisfactory results. When the number of animals to be treated is large the best method of combating ticks is to dip the animals in a suitable solution in the same way and with the same precautions as recommended in the case of mange. The following formula constitutes a satisfactory tick-killing solution (used in Queensland):—

Arsenious oxide 8 lb.  
Caustic soda 5 lb.  
Stockholm tar  $\frac{1}{2}$  gallon  
Tallow or oil (animal or vegetable) 4 lb.  
Water 400 gallons

*Directions.*—Mix from 8 to  $8\frac{1}{2}$  lb. of commercial arsenic (to contain 8 lb. arsenious oxide) in its powdered dry state intimately with 2 lb. of caustic soda, and while stirring add slowly upto 4 gallons of water. Heat to boiling point if arsenic has not properly dissolved. Then boil from 50 to 100 gallons water in a 400 gallons tank and add 2 lb. of caustic soda and 4 lb. of tallow (or oil), boil for about 15 minutes, then add slowly in a thin stream half a gallon of the best Stockholm tar. When the whole of the tar has been added, boil from 30 to 40 minutes, then add the arsenical solution and fill up the tank with water. It is advisable to test the safety of arsenical dips first on a few animals. Special care must be taken with such a dip when using it in a hot and humid atmosphere and if found necessary it may be further diluted so as to render it safe but not ineffective.

(v) *Lousiness.*—Three kinds of lice, namely *Haematopinus eurysternus*, the short-nosed cattle louse (Plate XIII, fig. 7), *Linognathus vituli*, the long-nosed cattle louse (Plate XIII, fig. 8) and *Trichodectes scalaris*, the biting louse of cattle (Plate XIII, fig. 9) are commonly met with on Indian cattle, particularly the young stock. Neglected and poorly-nourished cattle kept under insanitary conditions are more predisposed to infestation with lice. These parasites are specific for cattle and do not show any tendency to leave the host. Once the parasites and eggs become dislodged from the animal, they die within 7 or 8 days under most favourable conditions. The first two species of lice are known as the sucking lice because they puncture the skin and suck blood. They are about 2-3 mm. long. The biting louse, which is smaller and more common than the sucking louse, is commonly met with on the withers and around the root of the tail. The sucking louse, being more irritating, usually select such parts of the body where efforts of the animals to dislodge them cannot be successful, e.g. sides of the neck, brisket, back, inner surface of the thighs, on the head, and around the nose, eyes and ears. When the infestation is heavy they may be found on any part of the body.

All three species of lice feed on the tissues of their host and cause a great deal of irritation, evidenced by rubbing and scratching. In the infested parts scurf and even crusts of dried blood may be found and the hair may look coarse and erect. Heavy infestation results in emaciation and anaemia.

On careful examination one can find the parasite and its eggs (' nits '), the latter attached to the hairs.

For all the three species of lice the same method of treatment and control proves effective. This consists in isolating the affected animals and applying some parasiticide dressing. The dressing should be applied thoroughly and repeated twice or thrice with 15 or 16 days' interval, as some of the eggs may survive the first dressing and hatch in about 10-14 days, thus giving rise to a new generation of lice. When the number of animals to be treated is small, equal parts of cotton-seed oil and kerosene may be applied with a brush or two parts of kerosene emulsified with one part of milk and added to 8 parts of water may be applied by means of a spray pump all over the body, taking special care of the brisket, inside the thighs, ears, etc. If the weather is not cold the hair may be clipped before applying the dressing and burnt. When the number of animals to be treated is large, dipping is the best method of treatment; the lime and sulphur dip, as recommended for mange, may be used against lice, observing all the necessary precautions mentioned there. Attention should be paid to the proper cleanliness of the animals and the premises.

#### ACKNOWLEDGEMENTS

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# PHENOTHIAZINE—A REMARKABLY EFFICIENT ANTHELMINTIC

BY

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## Introduction

AS the result of a publication by E. F. Knipling in April, 1938, reporting the extraordinary efficiency of internal treatment with small doses of phenothiazine for preventing the development of hornfly larvae (*Lyperosia irritans*) in the faeces of cattle, a brief trial on pigs was carried out at this laboratory in order to ascertain whether the drug might not also possess a marked anthelmintic action.\* The experimental pigs used for this purpose were not very heavily infected and as the efficiency of the drug, as indicated by this trial, only proved to be between 0 per cent and 60 per cent nothing further was done until August, 1939, when news of the success of Australian workers reached us and a private communication was received from W. E. Swales giving an account of remarkably good results obtained by him in Canada for the elimination of worms from comparatively lightly infested sheep.

Fortunately, we were aware of a severe outbreak of parasitic gastritis within 20 miles of the laboratory at that time and still having in our possession a certain amount of phenothiazine left over from the previous trial in pigs were able to carry out an experiment forthwith, on heavily infected sheep. This trial produced a surprisingly good result and on reporting our findings to the Helminths Committee of the Agricultural Research Council it was decided that the Council should be asked to purchase some 500 pounds of the drug for the purpose of carrying out an extensive trial under farming conditions as speedily as possible.

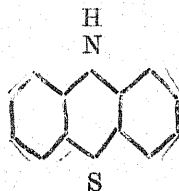
The symposium of which this paper forms part, deals with work carried out in various parts of the country and co-ordinated through the Agricultural Research Council.

## History of Phenothiazine

Phenothiazine is described by De Eds *et al.* (1938) as "a fine, smooth powder having a pale, lemon yellow colour, and is practically tasteless. When allowed to crystallize from solutions of alcohol, benzene, xylol, or toloul, flat leaf crystals with a soapy feel are formed. Phenothiazine melts sharply at

\*The phenothiazine used on this and on a later occasion was very kindly supplied by the Imperial Chemical Industries workers on insecticides at Jealott's Hill. At that time the drug was difficult to obtain and without their help our early trials might have been considerably delayed.

180° C., is soluble in fat solvents, but is practically insoluble in water. It has a molecular weight of 199·14, and the following structural formula :—



“When exposed to air and moisture, phenothiazine slowly undergoes spontaneous oxidation. It is the parent substance of a large number of dyes, the most important of which, as regards the present work, are the three thiazine dyes, thionol, Lauth’s violet, and methylene blue.” It was first prepared in 1885, by Bernthsen, but does not appear to have found any special uses nor was there any evidence of its parasitocidal properties until 1934 when Campbell *et al.* reported on its toxicity against culicine mosquito larvae. These trials showed it to be even more toxic than rotenone, its action proving to be quite effective down to a dilution of 1 : 1,000,000.

Smith, Munger and Siegler (1935) then tried it for the control of the codling moth and found it to be as effective as lead arsenate. Knippling (1938), however, was the first to report its marked action against parasites of domestic animals; he fed small amounts to cattle and found that whereas no toxic effects were observed, the development of hornfly larvae (*Lyperosia irritans*) was completely inhibited in the faeces for three days. Harwood, Jerstad and Swanson (1938) then tried phenothiazine in pigs and found a rather variable, but nevertheless marked action not only against ascarids but also against oesophagostomes. Harwood, Haberman and Jerstad (1939) later reported observations on eight sheep, in which they found marked anthelmintic action at a dose rate of 1 gramme per kg. body weight, against hookworms and nodular worms and also against *Haemonchus* and *Ostertagia*, the effect apparently being particularly marked against *Ostertagia*. Swales (1939) next reported results obtained in the treatment of ten lightly infected sheep; he perfected an effervescent tablet and found a dose of 0·6 gramme to 0·8 gramme per kg. body weight to have a very pronounced action against stomach worms, nodular worms and hookworms. Gordon (1939) in Australia, also published a note having found a dose of 0·2 gramme per kg. to have a variable efficiency against nodular worms, ranging upto 100 per cent. Along with Whitten (1939), he also published a short note reporting an efficiency of 100 per cent against *Haemonchus*. Concurrent with this publication came one from Roberts (1939), another Australian parasitologist, who reported observations on seven lambs in which a dose of 0·2 gramme per kg. was shown to have an efficiency ranging up to 94·7 per cent against nodular worms and more recently Swanson, Harwood and Connolly (1940) have reported trials in pigs in which they found doses of phenothiazine ranging from 5 grammes to 30 grammes, according to the size of the pig, to be very effective against nodular worms and ascarids, although less effective against light infections of ascaris than against heavy ones.

The results reported by the present authors concern more extensive trials on larger groups of animals most of which were actually suffering from helminthic disease at the time that the trial was carried out. Sheep and horses have been most largely used and results, almost without exception, have been extraordinarily good.

The phenothiazine used in these observations has been supplied by the Imperial Chemical Industries in four forms; (a) pure phenothiazine powder, having a purity of about 99 per cent; (b) phenothiazine powder mixed with a wetting agent to facilitate its mixture with water; (c) liquid phenothiazine, being a suspension of very finely powdered phenothiazine in water containing some dispersing agent, and (d) phenothiazine tablets, containing 5 grammes of the pure drug and a certain amount of excipient together with a substance that brings about a rapid disintegration of the tablets when placed in water.

## Experimental Data

### Sheep

#### ANTHELMINTIC EFFECT.

*Experiment No. 1.*—Eleven lambs, badly affected with parasitic gastritis were purchased from a flock among which fifty had just died from this disease. On reaching the laboratory they were divided at random into two groups, five being kept as controls and six receiving 15 grammes of the phenothiazine mixture recommended by Swales.\*

All of the lambs, controls as well as treated animals, were killed five days after the dosing had been carried out and worm counts were made on the stomach and intestinal contents. Results given in Table No. 1 show a very remarkable effect of the treatment in that no worms were recovered from the stomachs of the six treated lambs, mature *Ostertagia* and *Trichostrongylus* having been completely eliminated, whereas four of the five controls contained over 15,000 worms in the fourth stomach. The effect on worms in the small intestine was less striking but nevertheless demonstrated an efficiency of over 80 per cent.

*Experiment No. 2.*—When the outbreak of parasitic gastritis among the flock from which the above-mentioned eleven lambs had been purchased began to diminish, and deaths occurred less frequently, forty of the most severely affected of those still remaining alive were picked out for a further trial. They were divided at random into two equal groups, one of which received four weekly doses of phenothiazine, the other being kept as a control group.

\*Swales recommended a special mixture of phenothiazine of the following composition:—

Commercial phenothiazine (pulv.) . . . . .	80 parts
Starch (pulv.) . . . . .	9 "
Effervescent salts (sod. bicarb. 50 parts, dehydrated tartaric acid 45 parts) . . . . .	9 "
Dried ox gall . . . . .	2 "



Samples of faeces were collected for the purpose of making a composite count, and all of the lambs were weighed on each of the four occasions when dosing was carried out. Particulars are set out in Table III and demonstrate the effect of the drug during one month in bringing about an increase in weight of 2·2 lb. per head over and above the gain in weight of the untreated controls.

*Experiment No. 3.*—Concerns an outbreak of parasitic gastritis among a flock of 91 lambs of which 36 had already died before the experiment began.

The opportunity was afforded at the time that the diagnosis was made of examining the contents of the stomach of one of the lambs which had died of parasitic gastritis, and the infestation was found to comprise 20,750 *O. circumcincta*, 4,550 *T. axei* and 220 *H. contortus*.

The flock was then divided at random into two equal groups of 28 lambs ; members of one group being given a dose of 20 grammes of phenothiazine in the form of a draught, without the additional substances used by Swales and the other group were kept as an undosed control. Weights and composite egg counts were made on the first and on two subsequent occasions and are shown in Table IV.

The result obtained in this observation was very remarkable in that the dosed sheep made an increase of 12 lb. per head over and above the increase in weight of the controls during the six weeks of the trial, representing a total increase of 336 lb.

*Experiment No. 4.*—There had been several deaths from parasitic gastritis among this flock during an outbreak of the disease which had occurred some twelve weeks previously. A *post-mortem* examination carried out at that time had shown the following parasites to be present ; 23,940 *O. circumcincta*, 1,330 *T. axei* and 1,330 larvae in the stomach, and in the small intestine, 20,640 *T. vitrinus*, 1,290 *C. curticei*, 430 *C. oncophora*, 5,160 *N. filicollis* and 2,580 larvae. About 20 lambs had died and the remainder of the flock made a more or less satisfactory recovery before the test dosing was carried out on October 31st.

On this occasion the 30 poorest lambs were taken out of the flock of 400 ; these individuals were then selected in order, proceeding from the poorest to the best member of the group ; two out of every three were dosed with 30 grammes of phenothiazine in the form of capsules, and the third was kept as an undosed control : all were weighed. The results, which are given in Table V, show an average increase during the three weeks observational period of 2·1 lb. per head among the treated lambs, over and above the average increase per head of the undosed lambs.

*Experiment No. 5.*—K. D. Downham of the Harper Adams Agricultural College reported an observation on eight lambs suffering from parasitic gastritis in which during the first six days after treatment the four dosed lambs made an average gain of 5·5 lb. each, whereas the four undosed controls made an average gain of only 1·5 lb.

**EFFECT OF THE DOSING OF HEALTHY BUT INFECTED LAMBS.**—The following few observations were carried out on healthy lambs to ascertain whether the removal of a relatively slight infestation of parasitic worms, not sufficient to cause obvious symptoms of disease, might bring about an increased rate of growth.

*Experiment No. 6.*—This observation, carried out by K. D. Downham, concerned 20 healthy lambs, all born in March, and picked out from a well-managed flock that had been periodically dosed with copper sulphate and received frequent changes of pasture throughout the previous summer. The lambs were divided into two equal groups the members of one of which received a dose of 15 grammes of phenothiazine on December 6th.

A second weighing carried out three weeks later showed an average increase of 0·7 per head among the group of dosed lambs in excess of the increase among the controls.

*Experiment No. 7.*—Another collaborator, J. W. Ironside of the Midland Agricultural College, carried out a similar observation on ten healthy lambs, previously treated with copper sulphate and nicotine sulphate.

Results also showed a slight increase of weight in favour of the treated group which during four weeks gained an average of 10·4 lb. as compared with 9·8 lb. in the controls.

**EXPERIMENTS WITH SMALL DOSES.**—It was felt that the large size of the dose and the bulky nature of the drug threatened to be the most powerful factor operating against its general use in sheep and we, therefore, carried out a few trials to ascertain whether it might not be possible to secure adequate anthelmintic action with a much smaller dose. Results of the trials carried out on eight sheep are shown in Table VI and indicate that whereas doses of less than 3 grammes had no action, those of 3 grammes (0·08 grammes per kg. body weight) and over, produced a marked effect.\*

Some further evidence of the minimum effective dosage is given in Table IX, which suggests that amounts as small as 1·5 grammes and 1·75 grammes had some effect.

**DAILY DOSES OF PHENOTHIAZINE MIXED WITH THE FOOD.**—Doses of 2 grammes, 5 grammes and 10 grammes were tried in three pairs of sheep, the phenothiazine being given in powder form mixed with the food. The results, which are given in Table VII, show considerable effect even for the 2 gramme doses, the 10 gramme dose, however, producing the result more rapidly.

The sheep receiving the 10 gramme doses did not eat their medicated food at all readily but those on the smallest dose took it well; and suffered no ill effect whatsoever; the two sheep receiving 10 grammes daily, however, began to show signs of intoxication after the first week, dullness and inappetence being the principal symptoms, and on the thirteenth day one of them died, showing diarrhoeic symptoms for a few hours beforehand.

**TOLERANCE OF SHEEP.**—Apart from the daily dosing of 10 grammes no evidence of the slightest toxic effect had been observed in any of the therapeutic treatments of sheep; in an endeavour to obtain some idea of the toxic dose, therefore, four lambs weighing between 86 and 89 lb. received 40 grammes, 80 grammes, 200 grammes and 400 grammes of phenothiazine respectively, but without any signs of intoxication being produced.

The absence of result from the top dose seemed so surprising that it was repeated in another lamb of the same weight, but again produced no ill effect whatever.

\*It is possible that the dose of 1 gramme given to these sheep four days previously may have helped to bring about the observed result. This possibility also applies to the results obtained in the goats, which had also received small doses two or three days previously.

The lamb that died as a result of repeated daily doses refused food for the last day or two and showed a dark red diarrhoea before death ;\* this lamb was in a particularly poor condition when the treatment commenced, which may have had something to do with the fatal result ; a second one in better condition, however, also showed signs of intoxication although it survived 16 daily doses of 10 grammes. Two other lambs which received daily doses of 5 grammes each showed no such symptoms.

**SUMMARY OF OBSERVATIONS IN SHEEP.**—Extensive trials suggest that in doses of 15 to 30 grammes (0·5 to 1 gramme per kg. body weight) phenothiazine has a remarkably good anthelmintic effect in sheep, the efficiency of its action ranging round 100 per cent for worms in the fourth stomach and 80 per cent for most of the worms in the small intestine. Where used in actual outbreaks of parasitic gastritis its effect is reflected in very considerable gain in weight.

Less extensive trials suggest that doses as low as 0·08 gramme per kg. body-weight have a considerable anthelmintic effect although the action of larger doses is more reliable.

The tolerance of sheep is extraordinarily high, 400 grammes having been given without ill-effect ; a repeated daily dose of 10 grammes, however, produced a fatal intoxication in one instance.

Sheep will take 2 grammes to 5 grammes mixed with the food, a good anthelmintic effect being produced even by the smaller dose.

Administration is best carried out by means of 5 gramme tablets and a rubber-ended balling gun.† Given adequate assistance it is possible for one person using a balling gun to administer a 10-gramme dose to 160 lambs in an hour.

### Goats

*Experiment No. 8.*—The following observations were made on the effects of phenothiazine on a group of goats at the Ministry's laboratory, many of their number suffering severely from parasitic gastritis.‡

\**Post-mortem* findings in this sheep were as follows :—

Nasal passages filled with a catarrhal exudate of a pink colour, presumably due to the inhalation of phenothiazine powder, the mucous membrane over the turbinated bones and in the upper part of the trachea was of a dark reddish brown colour, but apparently not inflamed, the coloration presumably also being due to the formation of thionol from the drug. Bronchioles also contained much catarrhal exudate. Liver showed signs of fatty degeneration : the kidneys were of a dark red colour, the pelvis containing a brownish liquid. The fourth stomach showed a slight inflammation of the mucous membrane throughout its whole extent.

†In a private communication received since this was written, Dr. Robertson of the Aberdeen School of Agriculture claims to have dosed sheep with the liquid form of phenothiazine at the rate of four per minute. McEwen and Rowlands also preferred the liquid and as the tablets are very awkward to administer without the proper balling gun this form of the drug may be found to be the most generally useful.

‡These goats had been collected from various centres in Wales, and in Ireland, several of them being in a more or less advanced stage of parasitism on arriving at the Laboratory. On account of their different origins, therefore, the nature of their helminthic infections did not conform so nearly to one type as it would have done had they all come from one source.

Fifty-three goats were used for this experiment; 25 received one dose of phenothiazine in capsule form, varying between 10 grammes and 30 grammes according to the size of the goat, and 28 were kept as controls.

During the three weeks observational period the egg count among the 25 treated goats was reduced from an average of 5,080 to an average of 533, the egg count among the 28 controls falling from an average of 6,696 to 3,808 during the same period.

Several of the goats showed signs of intoxication for three or four days after the administration of these doses; they refused all food, looked dull, and were often seen to stand motionless in a corner of the stall with their heads hanging down.

As a result of the various origins of the experimental goats, and the small number of *post-mortem* examinations carried out, the results are less conclusive than they otherwise would have been, but they suggest that the drug acted particularly powerfully on *Ostretagia circumcincta* and on *Trichostrongylus axei*, less powerfully on worms in general in the small intestine and not at all on *Nematodirus filicollis*, *Moniezia* spp. and *Fasciola hepatica*.

The *post-mortem* results also suggest the 10 gramme dose to have been less efficient than the 20 and 30 gramme doses.

#### THE EFFECT OF SMALL DOSES IN GOATS.

*Experiment No. 9.*—In this experiment eight goats received gradually increasing doses of phenothiazine at intervals of four days, the doses ranging from 0.5 gramme to 12 grammes. The data which were collected are shown in Table II and indicate that doses up to 1½ grammes are without effect; a dose of 4 grammes appears to have had some effect, 6 grammes had a decided effect, and the 8 gramme dose appears to have been just as effective as the 12 gramme dose given later. In one or two instances, however, the small doses failed to expel a considerable number of worms, notably in goat 428. These results conform with those of experiment 8 and it may be concluded that doses of less than 20 grammes, although effective, cannot be relied upon to produce the maximum anthelmintic action.

**TOLERANCE OF GOATS.**—In view of the signs of toxic action seen in several of the treated goats a series of trials was carried out in order to assess the toxic dose. In the first trial six goat kids were treated with 10, 20, 30, 40, 50 and 60 grammes respectively, of phenothiazine, but without producing any ill effect. Eight adult goats were next treated with 50, 60, 70, 80, 90, 100, 110 and 120 grammes respectively, but again without producing any ill effect.

Only three days later when it became clear that there was a negative result from the previous administration a dose of 400 grammes was given to the goat that had previously received 80 grammes, but even this produced no noticeable effect whatever.

**SUMMARY OF RESULTS IN GOATS.**—Our observations suggest that at certain times goats may be less tolerant of phenothiazine than are sheep, but even at those times are good subjects for anthelmintic treatment with this drug. At other times they are able to withstand enormous doses, similar to sheep. Doses varying between 10 and 30 grammes reduced the egg count to one-tenth of what it was before treatment was carried out. A dose of 6 grammes reduced the infestation very considerably and an 8 gramme dose appeared to be in no way inferior to a 12 gramme dose.



**Cattle**

*Experiment No. 10.*—Concerned an outbreak of parasitic gastritis among seven Jersey calves in which two had just died at the time that the outbreak came to our notice. Egg counts made on samples of faeces collected from two dead calves on September 3rd gave a count of 3,900 and 1,500 eggs per gramme respectively, counts that are considered to be high for cattle. *Post-mortem* examination showed the presence of a pure infestation of *Ostertagia ostertagi*, 8,200 being recovered from the fourth stomach of one calf and 20,200 from the other.

Three of the five remaining calves were then treated with 25, 35 and 45 grammes of phenothiazine powder respectively, and the other two kept as controls. On September 13th the three treated calves were found to have improved in condition and to have recovered from the diarrhoea; one of the two controls, however, looked distinctly worse than on the previous occasion and so was given a dose of 30 grammes of phenothiazine. This animal died on September 20th, presumably having been beyond the hope of recovery at the time that treatment was given: *post-mortem* examination revealed the presence of only 200 *O. ostertagi* in the stomach and 600 in the small intestine. The remainder of the animals ultimately made a good recovery.

This observation appears to indicate the efficiency of 25 to 40 gramme doses of phenothiazine against *O. ostertagi* in calves.

*Experiment No. 11.*—Was carried out by W. J. Ironside of the Midland Agricultural College on six cattle which had previously received treatment with copper sulphate and nicotine sulphate.

The three treated cattle varied considerably in size but all received the same dose of 70 grammes of phenothiazine, as a result of which they were all very seriously upset and refused food for several days. One of the three, which was in a weak state at the time of dosing, became so ill that it was expected to die; but all ultimately made a good recovery.

The egg count per gramme of faeces in the treated cattle was reduced during the three weeks observational period from 800, 100 and 1,200 to 200, 0, and 0, respectively, whereas among the three controls the successive counts were 300, 1,300 and 700, respectively, at the first examination, and 400, 1,000 and 900 at the second.

**TOLERANCE OF CATTLE.**—The suggestion of susceptibility to toxic action given in *experiment 11* led to our carrying out one or two direct observations on this point, and on October 26th a dose of 1,000 grammes was given in the form of a drench to a 14-weeks-old calf, it being anticipated that the tolerance of cattle might approach that of sheep. Within an hour the calf lay down and would not rise; on the following day it was found to be lying on its side, the temperature having risen to 102.4°, at which point it remained. Great muscular weakness was noted on October 28th and 29th, on October 30th respiration was observed to be rapid, the pulse was weak, and death occurred during the afternoon.

*Post-mortem* examination showed extensive ulceration of the mucous membrane at the pyloric end of the stomach, and slight inflammation throughout the whole length of the bowel lining. The liver and lungs were congested; the kidneys appeared to be normal. Petechial haemorrhages were seen under



the pericardium. Some little time after exposure to air the whole carcase took on a red colour—due, presumably, to the oxidation of the thionol which is one of the products of phenothiazine in the animal body and is responsible for the redcoloration of the urine. Portions of tissue taken for sectioning also produced abright pink coloration in the fixative.

A second calf, 12 weeks old, and weighing about 150 lb., received a dose of 200 grammes on the same date, October 26th; on the 27th it was found to be lying down and refused food, during the following eight days it became increasingly weak and it died on November 5th.

At *post-mortem* examination ulceration of the abomasal mucous membrane was again seen, particularly at the pyloric end, the contents of the rumen were very fluid and there were signs of slight inflammation in the mucous membrane of the first, second and third stomachs. The intestines were also slightly inflamed. The liver was friable and yellowish in colour; the kidneys appeared to be enlarged but did not show pathological changes on macroscopic examination.

A third calf, 5 months old but weak and very small, weighing only 105 lb., was dosed with 85 grammes of phenothiazine in the form of a drench. On the following morning it was found lying down, sweating and showing signs of great distress, the abdomen being distended. As it appeared to be no better in the afternoon it was destroyed. At *post-mortem* examination the mucous membrane of the abomasum was found to be acutely inflamed, particularly at the pyloric end where ulceration had begun. Slight inflammation of the small intestine, extending into the caecum was also observed. The liver and kidneys were normal.

A fourth calf, also 5 months old, but well grown, weighing 273 lb., was treated with 100 grammes in liquid form but showed no untoward results whatsoever; it went on feeding and behaving in a perfectly normal way from the time that the dose was given.

**SUMMARY OF OBSERVATIONS IN CATTLE.**—Although two experiments—Nos. 10 and 11—gave indications of good results similar to those obtained in sheep, observations on the tolerance of cattle show them to be sensitive to the toxic action of the drug and suggest that a dose of something like two grammes per kilo may prove fatal to a 3 to 5-months-old calf.

It seems probable that the drug will prove less useful in cattle than in other ruminants.

### Horses

The remarkably good results obtained in the treatment of horses are recorded in Table VIII, *a*, *b*, and *c*, and represent data which we have been able to collect since September 15th, 1939, when the first trial was carried out.\*

The method of procedure has been to omit one or perhaps two feeds in order to increase the animal's appetite and then to offer the powdered drug mixed with a bran mash or with a little oats and treacle when, as a rule, the horse would take it voluntarily.

\*The trials in horses have been carried out with the kind co-operation of the following veterinarians and people associated with thorough bred studs: J. R. Barker, J. Bell, J. W. Bruford, F. J. Carless, P. Crosfield, K. D. Downham, C. C. Edmunds, J. Macarthur and E. B. Reynolds.

The method followed in carrying out the faecal examinations was as follows: First, an estimate of the number of eggs per gramme was made by applying the new McMaster technique,\* then the faeces were cultured at 26° C. and in eight days time the third-stage larvae separated by means of Baermann's apparatus; these were then differentiated into species and groups of species, and the original egg count divided proportionately.

These trials demonstrate a very remarkable efficiency for all of the strongyloid parasites of the large intestine, the adults at least, being completely eradicated as the result of the administration of a dose of between 30 and 60 grammes.

*Trichostrongylus axei*, presumably because of its different situation (in the stomach), is more resistant and in several instances appears to have been the only strongyloid parasite to withstand the treatment. *Ascaris* also responds to the treatment but *Anoplocephala*, data concerning which are not given in the table, did not.

**TOLERANCE OF HORSES.**—Horses appear to be very good subjects for treatment with phenothiazine, the large number of trials, data from which are set down in Table VIII, a, b, and c, having been carried out without any definite symptoms of intoxication although in several instances slight inappetence was observed about 24 hours after treatment.

**SUMMARY OF OBSERVATIONS IN HORSES.**—Phenothiazine at a dose rate of 0.16 gramme to 0.08 gramme per kg. body weight (30 grammes to 40 grammes for a hunter) is 100 per cent. efficient against red-worms. A dose of 20 grammes is also effective but less reliable, but a dose of 10 grammes produces no anthelmintic effect. *Trichostrongylus axei* does not respond well nor do *Anoplocephala* spp. but *Ascaris* is, as a rule, satisfactorily expelled.

Horses appear to tolerate this drug very well indeed, no clear instance of intoxication having been observed.

#### EFFECT OF PHENOTHIAZINE ON EGGS AND ON THE DEVELOPMENT OF INFECTIVE LARVAE IN THE FAECES

Examination of faeces from a calf which had received 45 grammes of phenothiazine on the previous day showed all of the eggs (some 1,100 per gramme) to be dead; the contents being opaque and shapeless and showing signs of degeneration.

Observations subsequently made on goats receiving small doses of phenothiazine showed that amounts too small to have any effect upon the adult worms were nevertheless sufficient to prevent the development of the larvae. The smallest dose employed was 0.25 gramme and as a goat passes something

\*The McMaster egg counting technique has been found, in our hands, to be as accurate as the dilution method previously in use at this Laboratory and is a quicker and more convenient way of working. We have introduced one slight improvement in that the 3 gramme sample of faeces is first mixed with water, centrifuged and the supernatant cloudy liquid discarded before a 66 per cent saturated salt solution is added and the count made in the special cell described by Gordon and Whitlock (1939). This preliminary washing not only allows a greater amount of light to make its way through the preparation but permits of the enumeration of the eggs of *Ascaris*, *Trichuris* and *Fasciola* and of lungworm larvae. These do not float to the top of the two-thirds saturated salt solution that is found to be the most useful medium for the enumeration of strongyloid eggs.

like 1,000 grammes of faeces during a day and the excretion of the phenothiazine in the faeces would be spread over three days it is obvious that the amount requisite for the prevention of larval development must be something quite small, presumably in the region of one part of phenothiazine in 8,000 of faeces.

The data from which this conclusion has been drawn are set down in Table IX.

Trials later carried out *in vitro* showed that a mixture of 1 : 100 or of 1 : 1,000 of phenothiazine powder in the faeces culture prevented the development of the larvae in a similar way. It was interesting to note, however, in an experiment in which the four treated goats were carrying a particularly heavy infection of *Muellerius*, that no effect whatsoever was produced on the larvae of this genus of lung-worms, living larvae being recovered from the culture in thousands.

An observation was next made to ascertain at what stage of larval development the action of the drug is most marked. Six 50-gramme cultures were made up for this purpose, half a gramme of phenothiazine being added to each at various stages during the culture period. When larvae were ultimately separated by means of Baermann's apparatus on the eighth day it was found that the drug had exerted its greatest influence on the eggs and early stages of development of the larvae. After the fifth day of development, *i.e.*, when the larvae had reached the infective stage, the drug had taken comparatively little action. Particulars of this observation are shown in Table X.

*Action in Vitro.*—Solutions of phenothiazine of various strengths were prepared in physiological saline, between 20 and 30 *Trichostrongylus* worms, freshly collected at *post-mortem* examination, being placed in each solution and kept at 37° C.

The results show that within 18 hours the saturated and half saturated solutions had begun to take effect although all of the test worms were not killed until they had been in these solutions for 66 hours. The quarter-saturated solution also appeared to take some effect but test worms in the eighth-saturated solutions, behaved in the same way as the controls in physiological saline.

As phenothiazine is described by chemists as "practically insoluble" this result would suggest a very potent anthelmintic action on the part of the very small amount of substance in solution.\*

### Phenothiazine as an Anthelmintic

#### DISCUSSION—MENTIONING DATA REPORTED IN OTHER CONTRIBUTIONS TO THE SYMPOSIUM†

##### GENERAL ANTHELMINTIC ACTION.

The extensive observations which are here reported on the anthelmintic action of phenothiazine in farm animals provide ample evidence of the great importance of the discovery of this parasiticide in the annals of helminth control by the use of drugs.

\*A. Eden of this laboratory has determined the solubility for us as being not greater than one in 50,000.

† "We" in this discussion refers to Taylor and Sanderson. Wherever reference is made to the work of other contributors to the symposium the name is given.

In common with other anthelmintics its mode of action remains something of a mystery, and its addition to the short list of really effective medicinal parasitocides cannot be regarded as the outcome of a scientific understanding of the principles by which their action is governed. The use of phenothiazine as an anthelmintic can be traced back through a series of rational trials to the first observation on its insecticidal properties, made by the workers in the United States Bureau of Chemistry and Soils, who on account of the lack of information at present available on the correlations between chemical structure and parasitocidal action were forced to carry out their investigations by a method of trial and error. As each new anthelmintic substance is found, however, fresh opportunity for study is provided, and it is not improbable that an investigation of some of the peculiar points concerning phenothiazine treatment may help to explain the mode of anthelmintic action.

The most outstanding peculiarity concerns the requirement for a large dose of the drug. Although it is practically insoluble in water we were able to demonstrate a definite parasitocidal action on adult worms *in vitro*, and a very marked toxic action on the eggs and preinfective larval stages of strongyloid worms in faeces, operating down to a dilution of about one part of the drug in 8,000 parts of faeces. (It was interesting to note, however, that *Muellerius* larvae were quite unaffected.) Knipling (1938) obtained similar results in his observations on the control of the hornfly in cattle when he found that the phenothiazine passing through the intestine of cattle for three days after dosing was sufficient to inhibit the development of hornfly larvae in the faeces.

These observations are suggestive of an extremely potent action of this drug since the actual amount in solution must be very small indeed, and it is surprising to find that rather large doses are requisite for full anthelmintic action. Swales (1939) considered it necessary to give 20 or 30 grammes to a sheep and Swanson *et al.* (1940) gave up to 30 grammes to a pig. Although our observations indicate the adequacy of a smaller dose it is nevertheless much greater than the tests *in vitro* would seem to indicate. Our results suggest that a dose of 5 grammes produces a good anthelmintic effect in sheep, and that 10 grammes approaches to the maximum anthelmintic effect. Ten grammes to a horse was clearly inadequate but 20 grammes, in some instances, proved to be 100 per cent efficient, although in others its action was much less marked; 30 grammes however produced the maximum result in almost every instance and it may be concluded from our results that for ordinary purposes, 10 grammes (0.3 grammes per kilo) is adequate for a sheep and 30 grammes (0.06 grammes per kilo) for a horse.

An interesting observation in connection with dosage was made at the Zoological Gardens, London, by G. M. Ververs who, in a private communication, reported the expulsion of large numbers of *Enterobius* from a gorilla as the result of the administration of only 0.5 grammes of phenothiazine.

There is, therefore, seen to be something unusual about the anthelmintic action of this drug in that the dose generally required, having regard to the insolubility of the drug, seems to be unnecessarily large. In an endeavour to explain this peculiarity we considered the possibility of the formation within the animal body, of some second substance, of much greater anthelmintic

*Worms recovered at post-mortem examination from six lambs dosed with 15 grammes of Secales phenothiazine mixture, and from five undosed controls*

No. of Lambs	Stomach.						Small Intestine.								Eggs per Gramme Faeces.					
	Total.	<i>O. circumcincta.</i>		<i>O. trifurcata.</i>		<i>T. axei.</i>	<i>H. contortus.</i>	Larvae.	Total.	<i>T. vitellus.</i>			<i>T. colubriformis.</i>	<i>C. curvicaet.</i>	<i>C. oncophora.</i>	<i>N. filicollis.</i>	Larvae.	<i>Moniezia.</i>	Before Dosing.	5 Days After Dosing.
Control Lambs	92	55,400	25,853	5,540	9,233	—	—	14,774	48,318	40,250	1,610	—	—	—	—	—	6,440	18	No count made	
	83	26,500	18,410	2,630	5,260	—	—	—	51,412	30,840	2,570	2,570	2,570	—	2,570	12,550	—	12	6,900	12,800
	84	15,100	11,325	1,510	2,265	—	—	—	15,500	6,200	3,875	—	—	—	—	4,650	775	—	11,500	5,300
	85	19,200	7,642	4,775	6,685	100	—	—	44,104	39,690	—	2,205	—	—	—	—	—	4	10,700	4,000
	90	2,100	2,100	—	—	—	—	—	13,002	9,750	1,300	680	—	—	—	1,300	—	2	1,600	1,600
Average		23,620	13,066	2,891	4,689	20	2,955	34,467	25,346	1,871	1,081	514	—	—	—	3,700	241	7	7,675	6,000
	86	—	—	—	—	—	—	—	1,004	400	—	—	—	—	—	—	800	4	4,200	200
	91	—	—	—	—	—	—	—	5,702	—	5,130	—	—	—	—	—	—	2	6,500	200
	89	—	—	—	—	—	—	—	9,407	2,350	—	470	—	—	—	6,500	—	7	8,700	600
	82	—	—	—	—	—	—	—	10,813	12,870	1,980	990	990	—	990	2,970	—	13	15,400	1,900
	87	—	—	—	—	—	—	—	3,004	2,100	600	—	—	—	—	300	—	4	5,600	300
	86	—	—	—	—	—	—	—	1,800	960	640	—	—	—	—	—	—	—	2,800	100
Average		—	—	—	—	—	—	—	6,755	3,113	1,391	243	260	—	—	1,028	100	5	7,200	550
Indicated efficiency per cent.		100	100	100	100	100	100	100	80.4	87.7	25.6	77.7	49.4	—	—	56.0	58.0	—	—	—



TABLE II (Experiment No. 9)

Showing the effect of small doses of phenothiazine in goats

No. of Goat.	Grammes of Phenothiazine Given.				Eggs per Gramme of Faeces.										Worms Found in Stomach.				Worms Found in Small Intestine.				Worms Found in Large Intestine.		Eggs per Gramme in Faeces.	
	10.4.40.	17.4.40.	22.4.40.	26.4.40.	27.3.40.	11.4.40.	12.4.40.	15.4.40.	17.4.40.	22.4.40.	20.4.40.	30.4.40.	4.5.40.	7.5.40.	H. contortus.	O. circumcincta.	O. trifurcata.	T. axei.	T. vitellus.	T. colubriformis.	N. filicollis.	T. ovis.	O. venulosum.	S. ovis.		
386	1	1	4	6	3,800	7,500	7,100	8,200	5,500	7,000	1,100	1,900	2,500	1,400	—	200	200	1,400	2,700	2,340	360	1	24	30	1,400	
387	1	1	4	6	1,100	1,300	300	600	700	1,400	200	200	500	200	—	—	—	200	1,200	1,200	—	1	8	—	200	
425	1	1	4	6	2,100	1,500	1,600	2,500	2,300	2,600	2,600	2,000	1,400	1,300	—	400	400	300	4,000	800	—	7	20	—	1,300	
430	1	1	4	6	1,200	3,400	3,500	2,200	5,700	6,600	1,500	600	200	100	—	100	—	100	500	200	200	10	—	—	100	
386	1	1½	8	12	300	100	200	300	600	100	0	0	0	0	—	—	—	—	—	—	—	22	—	—	—	
418	1	1½	8	12	4,400	4,500	5,700	6,900	1,600	1,700	100	100	100	0	—	100	—	—	400	—	—	—	—	—	—	
428	1	1½	8	12	2,400	4,600	8,000	9,400	7,000	2,400	2,100	3,200	4,300	4,200	100	3,173	—	20,627	11,873	1,827	—	15	—	2	4,200	
386	1	1½	8	12	2,000	2,300	1,300	3,800	2,500	3,300	800	700	600	500	900	2,000	—	2,700	1,200	1,500	—	48	185	308	541	

TABLE III (Experiment No. 2)

*Showing the effect of a 15 gramme dose of phenothiazine administered at the end of an outbreak of parasitic gastritis to a group of 20 lambs many of which had been severely affected.*

Dates of Dosings and Weighings.	20 Lambs dosed with Phenothiazine.			20 Control Lambs.		
	Average Weight in lb.	Average Increase in Weight.	Egg Count per gramme.	Average Weight in lb.	Average Increase in Weight.	Egg Count per gramme.
13-9-39	61.1		2,400	61.1		2,300
22-9-39	64.2	3.1		63.4	2.3	
29-9-39	65.4	1.2	500	64.4	1.0	1,400
13-10-39	70.8	5.4		68.6	4.2	

For particulars of worm infestation see Table I, the lambs referred to in Tables I and II belonging to the same flock.

Increase in favour of dosed sheep = 2.2 lb. per head in one month.

TABLE IV (Experiment No. 3)

*Showing the effect of a 20 gramme dose of phenothiazine administered towards the end of an outbreak of parasitic gastritis to a group of 28 lambs many of which had been severely affected. Species of Ostertagia were principally concerned in this outbreak.*

Dates of Dosings and Weighings.	28 Lambs dosed with Phenothiazine.			28 Control Lambs.		
	Average Weight in lb.	Average Increase in Weight.	Egg Count per gramme.	Average Weight in lb.	Average Increase in Weight.	Egg Count per gramme.
30-9-39	61.3		5,100	63.4		5,100
21-10-39	74.0	12.7	200	67.5	4.1	2,400
14-11-39	78.3	4.3	200	68.4	0.9	1,400

Increase in favour of dosed sheep in six weeks = 12 lb. per head, or a total of 336 lb.

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TABLE V (Experiment No. 4)

*Showing the effect of a 30 gramme dose of phenothiazine administered to lambs two months after the termination of an outbreak of parasitic gastritis, resulting principally from an Ostertagia infestation*

Dates of Dosing and Weighing.	10 Lambs dosed with 30 grammes Phenothiazine.			20 Control Lambs.		
	Average Weight in lb.	Average Increase in Weight.	Egg Count per gramme.	Average Weight in lb.	Average Increase in Weight.	Egg Count per gramme.
31-10-39	78.0		4,500	84.1		4,500
23-11-39	80.3	2.3	200	84.3	0.2	5,300

Increase in favour of dosed lambs = 2.1 lb. per head in three weeks.

TABLE VI

*Showing the effect of small doses of phenothiazine on a mixed infection, principally, Haemonchus contortus, in sheep*

No. of Sheep	Grammes of Phenothiazine given.				Eggs per gramme of Faeces.							
	19-4-40	23-4-40	27-4-40	1-5-40	12-4-40	20-4-40	23-4-40	27-4-40	1-5-40	6-5-40	10-5-40	15-5-40
1	1	1	3	5	12,000	16,900	22,900	2,000	800	300	400	300
2	1	1	3	5	2,600	5,900	4,900	6,300	700	100	700	700
3	1	1	3	5	800	2,900	2,500	2,800	300	200	400	400
4	1	1	3	5	500	300	1,300	800	800	1,200	700	400
5	1	2	4	6	7,600	3,900	5,700	7,700	700	100	300	1,000
6	1	2	4	6	2,200	2,400	7,200	1,800	500	600	500	300
7	1	2	4	6	1,000	4,100	3,900	2,100	500	100	200	800
8	1	2	4	6	800	1,500	3,500	1,600	600	200	700	400

TABLE VII

*Showing the effect of the repeated administration of small doses of phenothiazine to sheep*

Date.	Eggs per Gramme of Faeces in Six Experimental Sheep.					
	2 Gramme Doses of Phenothiazine Daily.		5 Gramme Doses of Phenothiazine Daily.		10 Gramme Doses of Phenothiazine Daily.	
	Sheep 1.	Sheep 2.	Sheep 3.	Sheep 4.	Sheep 5.	Sheep 6.
26-1-40	2,800	700	600	900	7,700	2,100
2-2-40	2,800	200	2,600	100	7,400	500
3-2-40	400	200	1,400	—	2,300	200
5-2-40	300	100	200	—	400	—
6-2-40	100	—	100	—	100	—
7-2-40	100	400	200	—	—	—
8-2-40	100	200	100	—	—	100
9-2-40	100	—	500	—	—	200
10-2-40	—	—	200	—	—	—
12-2-40	700	—	—	—	—	—
13-2-40	100	100	100	—	—	—
14-2-40	200	—	—	—	—	—
15-2-40	400	100	—	—	—	Died
16-2-40	—	—	—	—	—	
17-2-40	—	100	—	—	—	
19-2-40	—	100	—	—	—	
20-2-40	100	100	—	—	—	
21-2-40	—	—	—	—	—	
22-2-40	100	—	—	—	—	
23-2-40	—	—	—	—	—	
24-2-40	—	—	—	—	—	
25-2-40	—	—	—	—	—	
26-2-40	—	—	—	—	—	
27-2-40	—	200	—	—	—	
28-2-40	—	100	—	—	—	
29-2-40	100	100	—	—	—	

TABLE VIII

*Showing the effect of doses of phenothiazine ranging from 10 grammes to 25 grammes on strongyloid worms in horses*

Particulars of Horse.	Dose of Phenothiazine in Grammes.	Egg Count Before Treatment.						Egg Count 2 to 4 Weeks After Treatment.					
		<i>Ascaris.</i>	<i>Trichonema, etc.</i>	<i>S. edentatus.</i>	<i>S. equinus.</i>	<i>S. vulgaris.</i>	<i>T. axei.</i>	<i>Ascaris.</i>	<i>Trichonema, etc.</i>	<i>S. edentatus.</i>	<i>S. equinus.</i>	<i>S. vulgaris.</i>	<i>T. axei.</i>
6-year-old Hunter	10	—	350	115	5	30	0	0	864	216	0	120	0
	"	—	790	140	20	50	0	0	1,411	136	0	154	0
	"	—	98	80	40	18	0	0	178	12	10	0	0
	"	—	616	341	0	66	77	0	1,064	84	0	28	224
	"	—	803	275	0	11	11	0	1,920	336	0	144	0
4-year-old "	"	—	304	0	0	68	28	0	882	0	0	9	0
11-year-old "	"	—	97	1	0	0	2	0	837	9	0	64	0
5-year-old "	"	—	69	12	0	16	3	0	1,425	0	0	75	0
9-year-old "	"	—	1,302	0	0	208	0	0	255	12	0	21	12
10-year-old Thoroughbred	"	—	336	24	0	240	0	0	648	40	0	112	0
8-year-old "	15	—	372	108	12	606	12	0	40	3	0	57	0
3-year-old "	"	—	528	0	0	624	48	0	0	0	0	24	0
1-year-old "	"	—	462	14	0	224	0	0	0	0	0	+	0
2-year-old "	"	—	600	0	0	0	0	0	1	0	0	0	0
1-year-old "	20	—	400	0	0	0	0	0	100	0	0	0	0
	"	—	679	14	0	7	0	0	+	0	0	0	+
	"	—	1,170	18	0	612	0	0	8	6	0	186	0
Yearling Hunter	"	500	3,485	123	0	492	0	0	0	0	0	1	0
3-year-old "	"	—	1,216	32	0	352	0	0	0	80	0	320	0
	25	—	480	450	0	570	0	0	20	40	0	340	0
	"	—	480	450	0	570	0	0	20	40	0	340	0

— = less than 100 per Gramme. + = less than 1 per gramme. 0 = absent.



TABLE VIII<sub>B</sub>

Showing the effect of a dose of 30 grammes of phenothiazine on strongyloid worms in horses

Particulars of Horse.	Dose of Phenothiazine in Grammes.	Egg Count Before Treatment.						Egg Count 2 to 4 Weeks After Treatment.					
		<i>Ascaris</i> .	<i>Trichonema, etc.</i>	<i>S. edentatus</i> .	<i>S. equinus</i> .	<i>S. vulgaris</i> .	<i>T. axei</i> .	<i>Ascaris</i> .	<i>Trichonema, etc.</i>	<i>S. edentatus</i> .	<i>S. equinus</i> .	<i>S. vulgaris</i> .	<i>T. axei</i> .
Thoroughbred Foal	30	200	400	400	0	0	0	0	0	0	0	0	8
"	"	500	300	300	0	0	0	0	0	0	0	0	33
"	"	—	1,600	0	0	0	0	0	11	3	0	0	1
4-year-old Thoroughbred	"	—	252	24	0	0	24	0	3	0	0	0	3
2-year-old	"	—	495	0	0	5	0	0	0	0	0	+	+
2-year-old "	"	—	384	12	0	4	0	0	+	0	0	+	0
Yearling	"	100	640	0	0	144	16	2	0	0	0	0	0
Thoroughbred	"	—	720	32	0	48	0	0	0	0	0	0	8
"	"	200	1,581	85	0	34	0	300	0	0	0	0	0
"	"	300	990	33	0	77	0	0	0	0	0	0	0
"	"	—	344	28	0	28	0	0	0	0	0	0	0
"	"	—	156	87	0	36	21	0	0	0	0	0	1
"	"	—	920	0	0	70	10	0	0	0	0	0	2
"	"	—	640	0	0	160	0	0	0	0	0	0	2
"	"	—	700	0	0	0	0	0	0	0	0	0	0
"	"	—	192	4	0	2	2	0	0	0	0	0	5

— = less than 100 per gramme. + = less than 1 per gramme. 0 = absent.

TABLE VIIIc

*Showing the effect of doses of 50 and 60 grammes of phenothiazine on strongyloid worms in horses*

Particulars of Horse.	Dose of Phenothiazine in Grammes.	Egg Count Before Treatment.						Egg Count 2 to 4 Weeks After Treatment.					
		<i>Ascaris</i> .	<i>Trichonema, etc.</i>	<i>S. edentatus</i> .	<i>S. equinus</i> .	<i>S. vulgaris</i> .	<i>T. axei</i> .	<i>Ascaris</i> .	<i>Trichonema, etc.</i>	<i>S. edentatus</i> .	<i>S. equinus</i> .	<i>S. vulgaris</i> .	<i>T. axei</i> .
4-year-old Thoroughbred	50	—	500	0	0	0	0	0	+	0	0	+	0
"	"	—	1,598	34	0	68	0	0	0	0	0	4	0
-year-old	60	—	500	0	0	0	0	0	1	0	0	0	0
"	"	—	800	0	0	0	0	0	0	0	0	0	0
Cart colt	"	—	546	112	0	21	21	0	0	1	0	0	6
Thoroughbred barren mare	"	—	95	2	0	0	3	0	0	0	0	0	0
"	"	—	700	0	0	0	0	0	0	0	0	0	0
"	"	—	68	0	0	0	0	0	0	0	0	0	0
"	"	—	465	35	0	0	0	0	0	0	0	0	0
"	"	—	776	8	0	0	16	0	0	0	0	0	200
6-year-old Thoroughbred	"	—	1,064	84	0	28	0	0	0	0	0	0	7
4-year-old	"	—	1,920	336	0	144	0	0	0	0	0	0	8
11-year-old	"	—	882	0	0	9	0	0	0	0	0	0	0
5-year-old	"	—	837	9	0	54	0	0	0	6	0	0	0
9-year-old	"	—	1,425	0	0	75	0	0	0	1	0	5	0
Thoroughbred	"	—	980	0	0	20	0	0	0	0	0	0	2
"	"	—	990	0	0	10	0	0	0	0	0	0	0
"	"	—	207	48	3	39	3	0	0	0	0	0	2
Debilitated hunter	"	—	1,092	60	0	48	0	0	0	0	0	0	0
"	"	—	972	228	0	0	0	0	+	0	0	0	0
"	"	—	1,805	95	0	0	0	0	0	0	0	0	0
"	"	—	658	42	0	0	0	0	0	0	0	0	+
"	"	—	282	12	0	0	6	0	0	0	0	0	4
"	"	—	178	14	0	0	0	0	0	0	0	0	0

— = less than 100 per gramme.

+ = less than 1 per gramme. 0 = absent.

TABLE IX

*Showing the effect of small doses of phenothiazine in preventing the development of third-stage larvae in the faeces of goats*

Date.	Dose of Phenothiazine in Grammes.	Goat 388.		Goat 389		Goat 414.	
		Eggs per Gramme of Faeces.	Larvae per Gramme of Faeces.	Eggs per Gramme of Faeces.	Larvae per Gramme of Faeces.	Eggs per Gramme of Faeces.	Larvae per Gramme of Faeces.
7-3-40	0.25						
8-3-40		1,500	0	200	0	500	0
9-3-40		2,400	11	300	0	600	25
11-3-40	0.75	2,600	54	300	9	400	81
12-3-40		1,300	0	0	0	300	0
13-3-40	1.0	2,800	2	400	6	900	96
14-3-40		1,400	0	500	0	300	0
15-3-40	1.25	1,200	7	200	0	300	0
16-3-40		1,000	0	500	0	400	0
18-3-40	1.5	1,700	34	500	19	400	87
19-3-40		700	0	200	0	100	0
20-3-40	1.75	1,100	0	200	0	300	0
21-3-40		500	0	400	0	500	
25-3-40		500	2	400	0	200	0
26-3-40		500	86	200	130	300	144
27-3-40		600	51	100	19	400	140
28-3-40		700	170	300	112	700	92
1-4-40		800	124	400	132	600	204

TABLE X

*Showing the effect on the development of larvae of a mixture of 1 per cent. of phenothiazine in the faeces added at various stages during the culture period*

Date when 1 per cent. Phenothiazine was Mixed with Cultures made 15-12-39.	Number of Trichostrongyloid Larvae. Recovered 23-12-39	Date when 1 per cent. Phenothiazine was Mixed with Cultures made 15-12-39.	Number of Trichostrongyloid Larvae Recovered 23-12-39
15-12-39	0	19-12-39	30,640
16-12-39	2	20-12-39	53,200
18-12-39	5,680	Control	88,000

potency than that of phenothiazine, and for this purpose we administered 30 grammes to one goat, collected the faeces on the second day afterwards and

administered 30 grammes of the phenothiazine-containing faeces to a second goat heavily infected with parasitic worms. A filtrate of a watery solution of another 30 grammes of the same faeces was administered to a third goat, also heavily infected with parasitic worms. Observations on the egg output of the two goats that received the "medicated" faeces failed, however, to reveal any effect on the parasitic worms.

Following this observation we carried out two trials with oxidation products of phenothiazine produced within the animal body. The first of these was thionol\* which is the red dye that causes the red coloration of the urine in animals treated with phenothiazine and is the oxidation product of leucothionol which, after exposure of small portions of tissue to the air, is seen to be present in almost every part of the body of an animal that has received a large dose of phenothiazine. These substances form the reversible oxidation-reduction system thionol—leucothionol, similar to that of methylene blue—leucomethylene blue, well known to workers on clean milk. The second substance was phenothiazone which according to Gersdorff and Claborn (1938) is extremely toxic to goldfish.

Both of these substances were tested in doses of 0.25 grammes and 2 grammes in goats heavily infected with parasitic worms but without producing any anthelmintic effect whatsoever.

Nothing, therefore, was done to elucidate the reason for the necessity of giving much more phenothiazine than is required to produce a saturated solution in the contents of the intestinal tract in order to produce anthelmintic action,† but it seems not unlikely that some factor of importance for the understanding of the action of phenothiazine, and, possibly for the understanding of anthelmintic action in general, lies hidden in this one particular problem.

#### SPECIFICITY OF ANTHELMINTIC ACTION.

Our own results show that phenothiazine is particularly lethal to strongyloid worms,‡ its most pronounced and certain action being exerted on those in the large intestine of the horse, where, in adequate dosage, it can be relied upon to eradicate all of the adult forms. The species of *Strongylus*, however, appear to be slightly more resistant than are the smaller "red-worms," *S. vulgaris* being the most resistant of all.

Second only in efficiency is its action on the tricho-strongyloid worms in the fourth stomach of sheep where, in adequate dosage, it has proved to be 100 per cent efficient on several occasions; this high efficiency is, however, less reliable than on the red-worms in horses. Species of *Ostertagia* respond

\* The thionol and phenothiazone was kindly prepared for our use by Dr. Sexton of the Imperial Chemical Industries Laboratories at Blackley.

† The theory on which we are working at the present time is that it is necessary to maintain a saturated or nearly saturated solution of phenothiazine in the intestine for some considerable time in order to secure anthelmintic action and that to insure against the exhaustion of the reservoir of phenothiazine in the rumen, or in the large intestine in equines) it is necessary to give a large dose. *In vitro* observations appear to support this view.

‡ An interesting exception has been mentioned by Manson-Bahr, who, in a private communication, reported having found phenothiazine to be relatively ineffective against *Ancylostoma* although very effective against some other worms in the human being.

particularly well, *Haemonchus* appears to be less sensitive and *Trichostrongylus* and *Cooperia* occupy an intermediate position in this respect.

One interesting point which came to our notice was the very good action of the drug on *Trichostrongylus axei* in the fourth stomach of sheep—100 per cent frequently being removed—and its comparative lack of action on the same species when present in the stomach of the horse.

The action of the drug on worms in the small intestine of sheep is less pronounced than on those in the fourth stomach but is, nevertheless, better than that of any previously known anthelmintic. Apart from *Moniezia*, on which there is no action, *Nematodirus* appears to be the most resistant, but other species of trichostrongylid parasites in the small intestine may be regarded as giving a satisfactory response.

The results clearly show that there is no action upon *Fasciola*, nor upon *Anoplocephala*.

Action on ascarids is interesting in that it appears to be very good in the horse, moderately good in the pig and almost completely lacking in the dog, as shown by Montgomerie's results. This comparative response of ascarids to phenothiazine in these three kinds of hosts differs from their response to carbon tetrachloride as reported by Hall who found this drug to be more efficient against *Toxocara* and *Toxascaris* in the dog than against *Ascaris* in the horse.

#### ANTHELMINTIC EFFECT ON THE HOST.

The beneficial effect of treatment with phenothiazine has been very clearly demonstrated in some of the weighing experiments in which great gains have been observed as a result of the anthelmintic action of the drug. The most striking is described in *Experiment 3* in which one 20 gramme dose to each of a group of lambs resulted in the production of 336 lb. of mutton.

Observations carried out by McEwen and Taylor (to be reported later) and by McEwen alone on the Romney Marshes show significant gains in phenothiazine-treated sheep, over and above gains made by similar sheep treated with a mixture of nicotine sulphate and copper sulphate. A difference of 9 lb. per head among a group of 27 lambs was noted in one instance.

The results obtained by J. W. Ironside and K. D. Downham (reported in this paper) in lightly infected flocks are also interesting, showing a slight gain in favour of the treated groups. The trials were, however, not sufficiently extensive to establish this point and the result obtained by S. J. Menzies (also reported here) in comparing phenothiazine with copper sulphate suggests that the effect of copper sulphate may be equally good in instances of slight infestation. On account of the inadequacy of data, however, this point cannot be regarded as certain.

Very good results have been observed wherever it has been tried in horses.

The effect in goats and in cattle has also been very good, apart from the toxic action that has been observed in some instances.

#### TOLERANCE AND TOXIC ACTION.

Domestic animals in general appear to be extraordinarily tolerant to this drug and there is usually found to be an enormous difference between the anthelmintic dose and the toxic dose. Two sheep and one goat were actually



dosed with 80 times an effective anthelmintic dose without any signs of intoxication being produced. Horses appear to be only slightly less tolerant, Lapage, as reported in this symposium, having given as much as 500 grammes without producing marked symptoms, and a repeat dose of the same amount without causing a fatal intoxication; further data given by him, however, strongly suggest that 1,000 grammes produced a fatal result, although lesions found on *post-mortem* examination suggested that other pathological conditions may have been involved in the cause of death. Cattle, on the other hand, appear to be more sensitive to the toxic action of phenothiazine, 85 grammes having produced a fatal intoxication in a 105 lb. calf. The observations that we have been able to make concerning the use of the drug in cattle are few, but even in these animals the difference between the anthelmintic and the toxic dose appears to be considerably greater than it is with most, if not all other efficient anthelmintics.

Our observations have, however, revealed evidence of individual idiosyncrasy, or possibly of some special susceptibility associated with diet, so that we cannot yet write with certainty on the general safety of the larger doses. Our experience with goats provides an example of this point in that doses of 20 grammes and 30 grammes produced decided symptoms of intoxication at one time (shortly after the arrival of the animals at the Laboratory) and at a later date amounts as great as 400 grammes failed to produce any signs of even the slightest indisposition.

Some evidence of this occasional intoxication has also been seen in horses, slight constitutional disturbance having been shown on a few occasions, in the form of dullness and inappetence lasting for about 24 hours after the administration of the dose.

Observations made to determine the toxic dose in rabbits showed that a single dose of 6 grammes causes more or less marked constitutional disturbance and that 10 grammes is fatal, representing a toxic dose of 4 grammes per kilo.

The effect of repeated daily doses proved to be more marked; one rabbit died after only four consecutive daily doses of 2 grammes, another after seven daily doses of 5 grammes, a third after 17 daily doses of 1 gramme and a fourth was still surviving and showed little sign of intoxication after 28 daily doses of 0.5 grammes. The toxic repeated daily dose is therefore seen to lie somewhere between 0.05 and 0.1 gramme per kilo.

The principal lesion seen on *post-mortem* examination was that of acute gastritis, the condition being less acute, and progressing to ulceration confined to the pyloric end of the stomach, in the lower doses. In one or two instances, the small intestine was slightly inflamed, the liver sometimes showed signs of fatty degeneration and the kidneys were sometimes pale in colour and enlarged.\*

Further evidence of the greater toxicity of the repeated small doses over one large one was also obtained in *experiment No. 8* in which daily doses of only 10 grammes killed one sheep and caused a relatively severe constitutional

\* We have not yet had the opportunity of examining this and other material microscopically. Eddy *et al.* (1937), however, failed to find any injury to tissue after 295 consecutive daily doses to rats although the rats became stunted if the dose was sufficiently high.

disturbance in a second one. A similar result was obtained by Lapage (reported elsewhere in this symposium) who found repeated doses of comparatively small amounts of phenothiazine to be markedly toxic for pigs. Manson-Bahr, in a private communication to us, has reported that no toxic symptoms were noted in a human patient after a thrice-repeated daily dose of 8 grammes the anthelmintic effect of which was very satisfactory.†

#### ADMINISTRATION AND DOSAGE.

One great advantage of this drug is its comparative tastelessness, so that animals will take it voluntarily, mixed with the food, a point which is of particular importance in the treatment of the horse and is likely to add considerably to the extent to which phenothiazine will be generally employed. Although the suspicions of a well-fed animal, on being presented with a medicated feed, may be aroused to such an extent that he will refuse it, a fast of a few hours' duration usually overcomes the disinclination and the feed, which should be made as tempting as possible, is taken without further difficulty.

This method may also be employed in pigs, although Lapage points out elsewhere in this symposium that by mixing the drug with the pigs' food certain members of a group, which are more hungry, or less sensitive to the slight taste of phenothiazine, may get much more than the calculated dose and their companions much less. The method may also be used in the treatment of sheep, and the occasional medication of dry feed in this way is one of the possibilities for carrying out periodic dosing that is worthy of serious consideration. The results of *experiment No. 8* suggest that a dose of 2 grammes may be adequate for this purpose, although 5 grammes is better.

During the early part of our work it was feared that the bulky nature of phenothiazine, and the large size of the dose that was thought to be necessary would have a serious reaction on its general usefulness in sheep. In co-operation with Imperial Chemical Industries, however, two preparations have been made, a suspension containing about 40 per cent. by weight of phenothiazine, and a tablet, containing 5 grammes of phenothiazine along with a little excipient and a substance that brings about the rapid disintegration of the mass when placed in water, these have proved very good indeed.

Every opportunity has been taken to have the two methods of administration (in liquid and in tablet form) tried out by various people who have been using the drug in sheep, and opinion on their relative merits has been almost equally divided. The tablets are given by means of a rubber-ended balling gun and in our experience are to be preferred over the administration of a liquid where the dose to be administered is not a large one. On one occasion we were able to give a dose of 10 grammes (two tablets) to each of 120 lambs in only 45 minutes, without undue haste. Taking into consideration the greater safety of the tablet over the liquid method we are of the opinion that the tablet method is the better, although on account of the necessity of using a special balling gun the liquid may prove the more generally useful preparation.

Cattle can easily be treated by the administration of a liquid or of a draught made from tablets allowed to disintegrate in water or gruel.

† Manson-Bahr gave single doses of 30-40 grammes without ill effect except in one instance, when the 41-gramme dose produced sickness.

## GENERAL USEFULNESS.

The general advantages of this new anthelmintic may be listed as follows : (1) very high efficiency for certain parasites, (2) remarkable lack of toxicity, (3) lack of taste, (4) cheapness, (5) no previous preparation being required, (6) no subsequent purgation required. Against advantages the following two disadvantages only can be set : (1) its bulky nature, and (2) the appearance of the red dye thionol in the urine for some three days after the administration of phenothiazine.\*

The production of thionol in the urine is the only one of these points that calls for mention here, the others having been dealt with. It is, of course, always advisable to warn the owner of the treated animals about the appearance of this dye in the urine as it may easily be mistaken for blood and cause alarm where the warning has not been given ; apart from this, however, the consequent staining of the wool may be regarded by sheep farmers as a distinct drawback to the general use of phenothiazine, as thionol is a very fast dye and the stain is likely to remain on the wool for some time. As yet no suggestions have been made as to how this objection may be overcome.

There seems to be little doubt that phenothiazine will come into general use for the control of parasitic gastritis in sheep for which it has considerable advantages over any other known anthelmintic and it is felt that further trials will establish its usefulness for the same disease in cattle, in which animals parasitic gastritis is still without a satisfactory treatment. Australian and American workers have demonstrated its usefulness for the expulsion of oesophagostomes from sheep and some data collected by McEwen and Taylor but not published here, have shown a marked action on *Chabertia*. Its greatest use may, however, prove to be in the treatment of strongylosis in equines, where it can be relied upon to exert a 100 per cent. efficiency. If the safety of this treatment is also taken into consideration there seems to be every reason for its frequent and regular employment in horses and it is by no means too extravagant to suppose that by regular dosings every two or three weeks it may prove possible to eradicate the infestation from certain environments, a procedure which could not previously have been contemplated with hope of success.

## Summary

Phenothiazine is shown by the tests reported in this symposium, on some 150 horses, to be remarkably efficient against the strongyloid parasites in the large bowel of these animals, a dose of 30 to 40 grammes proving to have an absolute efficiency of 100 per cent for adult worms.

The ease of administration and the safety of the drug are such that the eradication of red-worms from well-managed studs now appears to be a possibility.

Phenothiazine is also remarkably efficient against the stomach worms associated with parasitic gastritis in ruminants, as demonstrated by the several

\* The danger should also be kept in mind of the coloration of the carcase, and particularly the kidneys with thionol as the result of giving a large dose to lambs up to three days before slaughter.

observations involving some 300 to 400 sheep, 70 to 80 goats and a dozen cattle. In the small intestine of these animals it is less efficient but removes about 80 per cent of the trichostrongylid parasites there, with the exception of *Nematodirus*, on which it exerts very little action.

There is no action against *Fasciola* nor against the anoplocephaline cestodes *Moniezia* and *Anoplocephala*.

Ten grammes appears to be an adequate dose in sheep or goats although doses of 20 to 30 grammes are more likely to produce the maximum effect.

Cattle respond well but are much less tolerant of the drug than are sheep or horses.

Experiments on pigs and dogs have been few but it is clear that although the drug acts well on ascarids in the horse its action against these parasites is uncertain in the pig and is absent in the dog.

The anthelmintic effect noted on sheep that have been more or less heavily infested with stomach worms has been extraordinarily good and, where comparisons have been made, has generally been superior to that of copper sulphate and nicotine sulphate.

The tolerance of sheep and goats is very high, 400 grammes causing no ill effect: horses withstand up to 17 times the anthelmintic dose.

Some evidence of idiosyncrasy has been observed in that a group of goats showed some sensitivity to the drug on one occasion and on a later occasion proved to be very resistant to toxic action.

Repeated small doses are considerably more toxic than are occasional larger doses, the minimum toxic single dose being about forty times as great as the minimum toxic dose when repeated daily.

The tasteless character of phenothiazine is a great advantage in that horses will take it voluntarily in the food; pigs and sheep may also be dosed in this way.

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# COMPARATIVE TESTS ON THE TREATMENT OF LAMBS WITH PHENOTHIAZINE AND WITH COPPER SULPHATE AND NICOTINE SULPHATE

BY

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(Reprinted from *The Veterinary Record*, Vol. 52, No. 36, September 7, 1940.)

## Experiment 1.

THIS experiment was carried out in co-operation with Dr. E. L. Taylor and the experimental animals were purchased and maintained at the expense of the Veterinary Laboratory, Ministry of Agriculture, Weybridge.

A flock of 123 lambs, seven to eight months old, was placed in experiment in November, 1939. The animals were divided into three groups, each containing 41 lambs, and they were kept throughout the experiment on a limited range of permanent pasture that had been heavily stocked with sheep.

Group 1 was treated every two weeks with 30 grammes of phenothiazine.

Group 2 was treated every two weeks with 20 c.c. copper sulphate and nicotine sulphate.\*

Group 3 was left untreated as a control group.

The lambs were weighed every two weeks. Treatment began on December 11th, 1939, and the lambs were dosed ten times, the last treatment being given on April 29th, 1940. The final weighing was made on May 20th, 1940. The lambs were sold by public auction on June 7th, 1940.

TABLE I

*Showing Death Rate and the Sums for which Animals were Sold*

Group.	No. in. Experiment.	No. Died.	No. Survived.	No. Sold.	Amount.
1	41	10	31	30	£ s. d. 52 17 6
2	41	8	33	31	51 5
3	41	21	20	19	28 10 0

\* The mixture used contained approximately 5 per cent copper sulphate and 5 per cent "nicotine sulphate 40 solution."

TABLE II  
*Showing the Mean Weights*

Date Weighed.	Group 1.	Group 2.	Group 3.
11-12-39	71	72	74
22-12-39	68	69	71
8-1-40	68	70	69
5-2-40	62	63	61
19-2-40	64	64	61
4-3-40	61	59	58
18-3-40	63	59	58
1-4-40	65	61	59
15-4-40	69	65	61
29-4-40	74	71	68
20-5-40	83	82	75

In Table 2 are shown the mean weights of the three groups at various intervals after the commencement of the experiment. It may be stated that from March 18th onwards, the mean weight of the lambs in Group 1 was significantly higher than that of the lambs in Group 3. The animals in Group 2 occupy an intermediate position between Groups 1 and 3, and this suggests that phenothiazine is the better of the two anthelmintics.

**Experiment 2.**

In this experiment the dose of phenothiazine was reduced to the smaller and more convenient one of 10 grammes; the 20 c.c. dose of copper sulphate and nicotine sulphate was retained. Fifty-four of the poorest lambs, in a flock showing evidence of parasitic gastritis and enteritis, were selected and divided into two approximately equal groups. The lambs in Group 1 were given phenothiazine and those in Group 2 were given the copper sulphate and nicotine sulphate mixture. No controls were kept. The lambs were first weighed and treated on March 18th, 1940. Altogether the lambs were dosed four times at fortnightly intervals and on each occasion they were weighed. Their weights were taken for the fifth and last time on May 20th, 1940.

TABLE III  
*Showing the Mean Weights of the Lambs*

Date Weighed.	Group 1.	Group 2.
18-3-40	71	67
1-4-40	72	68
15-4-40	77	72
29-4-40	83	78
20-5-40	96	87

It may be stated that at the last weighing the difference of 9 lb. between the mean weights of the two groups was found to be statistically significant. The experiment therefore suggests that phenothiazine is the better anthelmintic.

**Experiment 3.**

Observations were made on three flocks of 11 to 12 month-old lambs belonging to the same owner. Each flock of lambs was divided into two equal groups and one group was treated with phenothiazine while the other received copper sulphate and nicotine sulphate. The dose of the former was 10 grammes and the dose of the latter 20 c.c. The animals were not weighed and the effects of the two different treatments were compared from the appearance of the animals approximately three weeks after treatment stopped.

Flock 1 consisted of 86 lambs ; treatment began in March and the animals were dosed three times at intervals of three to four weeks.

Flock 2 contained 45 lambs ; treatment began in April and the lambs were dosed twice.

Flock 3 contained 30 lambs and they were dosed in the latter part of April.

The lambs were inspected in May, between two to three weeks after the last treatment had been given. Each flock was divided into its two groups that were held for examination in adjacent sheep pens. Without knowing the treatment given to each individual group I selected the better group in each flock and in all three cases this proved to be the group that had been treated with phenothiazine. The superior condition and bloom of the lambs that received phenothiazine was most marked in the flock that had been treated for the longest period and had been dosed three times. In the flock that had been treated on one occasion only, the difference was least obvious. In each case my selection of the better group was confirmed later on in the same day by a shepherd who was not aware that the animals had been treated. These observations again suggest that phenothiazine is the better anthelmintic.

**Summary**

Comparative tests made on five flocks of lambs indicate that phenothiazine is a more effective anthelmintic than copper sulphate and nicotine sulphate.

\* \* \* \* \*

# PHENOTHIAZINE IN PARASITIC GASTRITIS

BY

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(Reprinted from *The Veterinary Record*, Vol. 52, No. 36, September 7, 1940.)

THE results of small scale experiments in Australia showed that phenothiazine was of value in treating parasitic gastritis in sheep. The following is the account of an experiment carried out in North-Wales. In no case, however, could the sheep be regarded as typical cases of acute parasitic gastritis.

## FARM A.

Eleven wether (Welsh) lambs—"culls"—showed symptoms of scouring, extreme emaciation, poor fleece and increased lacrimation. They had been, grazing on a permanent pasture and had ceased thriving from the end of August. They had been dosed at intervals with proprietary worm pills.

A *post-mortem* examination of an emaciated lamb showed the following worms to be present:—

4th Stomach—1,600	{	<i>Haemonchus contortus</i>	.	.	100
		<i>Ostertagia</i>	.	.	1,100
		<i>Trichostrongylus</i>	.	.	400
Small Intestine—15,200	{	<i>Haemonchus contortus</i>	.	.	7,296
		<i>Trichostrongylus</i>	.	.	5,472
		<i>Cooperia</i>	.	.	2,432

The lambs were divided into two groups; the six poorest were dosed with 20 grammes phenothiazine (tablet form) crushed and suspended in water and drenched on December 1st. Five lambs acted as controls, receiving no treatment. All the lambs were weighed prior to dosing and two composite faeces samples consisting of the faeces of each individual lamb in each group were taken. The results are set out below:—

Group.	Date.	Average Weight.	Average Loss in Weight.	Average Egg Count.
Dosed . . . . .	1-12-39	34.7 lb.	0.4 lb.	2,100
	20-12-39	34.3 "		400
Undosed . . . . .	1-12-39	44.2 "	1.8 "	1,700
	20-12-39	42.4 "		600

## FARM B.

This is an upland farm and the flock consisted of 73 ewes, 2 rams, and 15 lambs. There were no obvious clinical symptoms, except those of poor thriving, the condition being due, in part at least, to the poor pasture. After weighing a fair proportion of the dosed and undosed ewes and lambs and obtaining

two composite faeces samples, 53 ewes, 2 rams and 10 lambs were drenched with 20 grammes phenothiazine, 20 ewes and 5 lambs being left as controls. The results are shown in the table below :

Group.	Date.	Average Weight.	Average Loss in Weight.	Average Egg Count.
Ewes	Dosed . . . . .	1-12-39 29-12-39	59.0 lb. 55.1 "	3.9 lb. 300 Nil
	Undosed . . . . .	7-12-39 29-12-39	60.0 " 54.0 "	6.0 " Nil Nil
	Dosed . . . . .	7-12-39 29-12-39	43.6 " 39.8 "	3.8 " 300 Nil
	Undosed . . . . .	7-12-39 29-12-39	42.4 " 37.4 "	5.0 " 100 400

Two further experiments were carried out on a small group of lambs at the laboratory. Five wether lambs that previously grazed upon a permanent pasture on the College Farm, Aber, were treated. Particulars of dosage, egg counts and weights are set out in the accompanying table and appear to indicate a marked beneficial result from the use of phenothiazine.

Five wether lambs from a farm where losses had been comparatively heavy were sent to the laboratory. A *post-mortem* examination on one lamb that died had revealed the presence of 1,200 nematodes in the fourth stomach and 1,100 in the small intestine. Two weeks later 20 grammes phenothiazine was administered to the four lambs. A *post-mortem* examination on one of them which died shortly afterwards showed the complete absence of parasitic worms, eggs disappeared from the faeces of those remaining alive and their general bodily condition became greatly improved.

### Conclusion and Summary

- (1) Phenothiazine is a very efficient anthelmintic in sheep, providing the dose is sufficiently high—20 grammes or more.
- (2) Phenothiazine is well tolerated, 60 grammes having been given to a 50 lb. lamb without ill effects.
- (3) Phenothiazine is best administered in liquid form, with the aid of a dosing syringe.
- (4) Before phenothiazine is administered owners should be warned of the red colour that it produces in the urine.

\* \* \* \* \*



TABLE: Showing the Effect of Phenothiazine in Reducing the Worm Infestation in Five Experimental Lambs.

Date.	L. 1.		L. 2.		L. 3.		L. 12.		L. 17.	
	Treat- ment.	Weight in lb. E. P. G.	Treat- ment.	Weight in lb. E. P. G.	Treat- ment.	Weight in lb. E. P. G.	Treat- ment.	Weight in lb. E. P. G.	Treat- ment.	Weight in lb. E. P. G.
7-11-39		50 1,100	10 grammes 35 2,000 Phenothiazine		10 grammes 40 600 Phenothiazine		15 c.c. 45 700 Nicotine mixture		15 c.c. 45 5,100 Nicotine mixture	
15-11-39		41	43 700		Died 13-11-39*		49 1,900		51 1,300	
29-11-39	18 grammes 40 1,100 of Phenothiazine		1-12-39 43 600 20 grammes Phenothiazine				50 600		51 700	
13-12-39		45 300	43 1,000				51 400		49 1,000	
28-12-39		46 100	46 nil				44 300	21-12-39; 48 20 grammes Phenothiazine	nil	
12-1-39	11-1-39; 52 nil 60 grammes Phenothiazine		50 nil				11-1-39; 48 50 grammes Phenothiazine	nil	51 nil	
26-1-39		54 nil	53 nil				49 nil	nil	57 nil	
6-2-39		56 nil	50 nil				50 nil	nil	60 nil	
Increase in weight over experimental period	6		15				5		15	

\* Post-mortem examination showed no worms in abomasum only 300 in small intestine.

## ABSTRACTS

**Study on the mortality rates of calves in 335 herds in England and Wales (together with some limited observations for Scotland).** R. LOVELL AND A. B. HILL. (1940). *J. Dairy Res.* 11, 225.

PREVIOUS observations by two independent observers on herds in Ayrshire (Scotland) showed an annual mortality of 20-22 per cent with 27 per cent in spring and 8 per cent in autumn calves.

In the present survey the total number of live births reported in 335 herds (14,000 cows) in England and Wales during the years 1936-37 was 25,209 (12,665 male and 12,544 female); the mortality per 100 livebirths was 4.4 male and 5.5 female (the number for bull calves is low owing to disposal at an early age to butchers); the number of stillbirths was 4.7 per 100 total births; the number of abortions was 6.5 per 100 pregnancies. The total losses (deaths, stillbirths and abortions) were 14.3 per 100 pregnancies.

The corresponding figures for 47 herds (1,750 cows) in Scotland were: mortality per 100 livebirths, 4.0 male and 11.4 female; stillbirths 2.9 per 100 total births; abortions 5.2 per 100 pregnancies; and total losses 15.1 per 100 pregnancies. In both surveys roughly one in seven pregnancies failed to produce an adult animal.

In determining the rates of losses in separate herds, half the herds had less than 4 per cent of total losses on the average; one herd in five lost more than 10 per cent of its liveborn calves, one herd in ten had more than 10 per cent of pregnancies ending in stillbirths and one herd in five more than 10 per cent end in abortions.

With regard to the seasonal incidence of mortality, the Scottish rates for female calves were 12.2 per cent for the first half and 3.7 per cent in the second half of the calendar year; the English rates for the first and second halves of the year were: males 5.2 and 3.7, females 6.2 and 5.0 per cent. The high mortality rate in Scotland is entirely due to large numbers in the first half of the year. Factors inimical to calf life have a greater influence in this period but these factors do not affect the products of conception in utero.

There was no great variation in mortality in the different regions from which the data were collected, except for a suggestion of a higher rate in Northern England. The mortality rates are also not seriously affected by size of herd. The average female death rate in herds under 20 was 6.7, and in herds over 20, 5.4 per cent.

Colostrum was suckled in 71, given by bucket in 27 and not given in 2 per cent of 240 herds; the relative death rates for these classes were: male 100, 119, 131, and female 100, 124 and 141 respectively, the differences being significant for the first two groups of females only. In methods of feeding calves, more calves died when fed diluted milk by bucket (5.8) than when suckled (4.8) or bucket-fed undiluted (4.7); statistically the differences are not significant.

Nearly half the deaths of female calves took place in the first week of life and three quarters in the first month. [W. L. D.]

**Microbiology of silage made by the addition of mineral acids to crops rich in protein. II. The microflora.\*** A. CUNNINGHAM AND A. M. SMITH. (1940). *J. Dairy Res.* 11, 243.

THE addition of mineral acids to green vegetable matter so as to reduce the pH below 4.0 successfully preserves even highly nitrogenous materials such as leguminous crops. This is the A. I. V. process of silage making. The acid treatment however does not completely inhibit the growth of bacteria and other micro-organisms. The organisms which can grow in A. I. V. silage must be those which can initiate growth below pH 4, and it was anticipated that such silage would contain some unusual types.

\* The first paper of this series appeared in *Zbl. Bakt.*, 1939, II, 100, 394-408, not at present able to be consulted.

The microflora of this form of silage consisted mostly of lactic acid bacteria—lactobacilli, streptococci, micrococci and sarcinae, together with yeast and yeast-like organisms. In the lactobacilli homo- and heterofermentative types were represented, e.g. *Lactobacillus plantarum* in the former and *L. brevis* in the latter group.

The homofermentative streptococcal types belonged to the *Streptococcus lactis* group and the heterofermentative forms were identified as *Leuconostoc mesenteroides*. The types of micrococci and sarcinae isolated were not identified but were shown to possess characteristics not usually associated with these types of organisms, namely the formation of high concentrations of lactic acid and some carbon dioxide.

Freshly ensiled fodder contained motile lactobacilli, streptococci and micrococci but the majority of the lactobacilli and sarcinae were associated with older samples (cf., the microflora of cheese at different stages of ripening).

The characteristics which proved to be valuable for the differentiation of the organisms were ability to produce carbon dioxide, amount of lactic acid formed and the ratio of lactic to acetic acid. [W. L. D.]

#### Passage of carotenoids from food to milk in the cow. The fate of lycopene.

A. E. GILLAM AND S. K. KON. (1940). *J. Dairy Res.* 11, 266.

IN order to find out whether the tomato pigment, lycopene, an isomer of carotene, can pass from the food into the milk of the cow, three different cows were fed with 1,500 g. of tomato purée daily (in four fractions) and the resulting milk fats were examined for lycopene. When the fats were submitted to examination by careful chromatographic adsorption, no evidence of the presence of the pigment in the unsaponifiable matter of the butterfats was detected.

It was concluded that although the cow normally absorbs  $\alpha$ - and  $\beta$ -carotene and certain oxidation products of these substances from its food, it completely excludes the isomeric and very similar lycopene which was found in considerable quantities in the faeces after tomato feeding.

The examination of the carotenoids of several colostrum fats also provided no evidence of the presence of lycopene. [W. L. D.]

#### Subclinical staphylococcus mastitis in herds free from streptococcus mastitis, and its effect upon milk composition.

P. M. F. SHATTOCK AND E. C. V. MATTICK. (1940). *J. Dairy Res.* 11, 311.

IN the milk of herds free from streptococcal udder infection, changes in chemical composition and the pH of the milk have been found to accompany the presence of staphylococci.

In schemes for the control of mastitis in dairy herds based on the detection of *Str. agalactiae* in the milk, cases of infection by staphylococci are missed because their growth is suppressed on Edwards' crystal violet blood agar. It is known that clinical cases of staphylococcus mastitis causing serious disturbances in milk yield and composition are rare; the subclinical type may however be present to a considerable extent and remain unsuspected.

The present work shows that in cows free from streptococcus mastitis (428 samples) staphylococci were present in 21 per cent of the cases and no clinical case was observed even with careful recording of all abnormalities of milk and udder. The changes in milk composition due to this form of infection are similar to those occurring in cases of streptococcus mastitis, namely, a lowering of the 'casein number' below 78 and a rise in pH above 6.6. In 39 analyses, 10 samples with counts of *Staph. aureus* of from 150 to over 2,000 per ml. had a pH of 6.80-6.95 and a casein number of 68-77, the changes being greatest in samples with high counts.

It was established that changes in milk composition occur with staphylococcus infection but no evidence was available that such changes affected the behaviour of milk in the manufacture of products. [W. L. D.]

**Component acids and glycerides of some Indian ox depot fats.** T. P. HILDITCH  
AND K. S. MURTI. (1940). *Biochem. J.* **34**, 1301.

FOUR widely selected ox depot fats were examined, viz. those of a cow and bullock in Bombay, that of a cow in Calicut and a mixed sample from both sexes from Calcutta. The individual samples were probably taken from more than one animal. They were reasonably regular in melting point of fat and of fatty acids but the iodine value of the Bombay fats was much lower (26) than that of the other samples (31). The samples had developed free acidity before analysis and the neutralised samples only were analysed for component fatty acids and component glycerides by the methods usually adopted at the Liverpool laboratories, after preliminary fractionation by crystallization from acetone at low temperatures.

Oxen in the Bombay area are fed on grass, *jowar*, wheat, bran and cottonseed while the main diet of the Calcutta and Calicut animals was grass and paddy straw. This difference in food was the major cause of the different compositions of the depot fats.

All the depot fats examined were much more saturated than those of European and American animals, and contained only 27-33 per cent of unsaturated acids (mainly oleic) (English, 35 per cent oleic). The stearic acid content did not exceed 26-28 per cent; the palmitic acid was generally higher than the  $30 \pm 3$  per cent hitherto considered characteristic of ox fat. In the sample from the Bombay cow, palmitic formed over 40 per cent of the total fatty acids. The presence of larger amounts of palmitic caused the melting point of the fat and the solidifying point of the fatty acids to be the same as for ordinary ox fats.

The component glycerides of two cow fats studied in detail were found to conform in structure to other ox depot fats. The larger amounts of palmitic acid was reflected in increased amounts of dipalmito glycerides, but the amounts of monopalmito glycerides were comparable to those in other ox depot fats after allowing for the more highly saturated nature of the fats.

[W. L. D.]

**Studies on the secretion of milk fat. 3. Effect of thyroxine administration on the blood lipoids and on the nature of milk fat.** J. A. B. SMITH  
AND N. N. DASTUR. (1940). *Biochem. J.* **34**, 1093.

PREVIOUS experiments by others have shown that the feeding of dried thyroid gland to cows or the intramuscular injection of thyroxine raises the milk yield and the percentage of fat and solids not fat in the milk, and lowers the fatty acid but raises the sugar content of arterial blood.

Four cows in the present investigation were injected intramuscularly with 10 mg. thyroxine daily for a 10-day period; the milk was sampled and weighed before, during, and after this period. The milk yields of all cows were increased greatly during and for 3 days after the thyroxine period; of the milk constituents only the fat showed a slight increase due to thyroxine treatment in 3 cows while a small rise in lactose was evident in the milk of the fourth animal. These results generally fail to confirm previous findings of raising the solids not fat content of milk by thyroxine treatment.

A detailed study of the daily change in the composition of the fat was made (Reichert and iodine values). It is unfortunate to observe that the composition of the fats from the milk of 3 cows was abnormal in the control period (Reichert, 18 to 26, iodine value 44 to 54). The fat from one of these cows, examined in detail showed a Reichert value of 17.2 and an iodine value of 48.4 after thyroxine treatment, but values of 16.8 and 46.5 respectively 15 days after the end of that period. These butterfats had high oleic and palmitic but low butyric, myristic and stearic acid contents.

The sugar content of blood plasma was increased by some 10-26 per cent and the main lipid constituents decreased by 10-20 per cent during the period of thyroxine treatment.

[W. L. D.]



**Purebred and crossbred pigs. Comparison of rate of growth and economy of gains.** F. B. HEADLEY (1940). *Bull. Nevada Agric. Expt. Sta.* 153.

**H**YBRID vigour in both plants and animals is a well-known phenomenon. In the case of pigs, faster and more economical growth are practical manifestations of this vigour. Crossbred Duroc-Poland China pigs were studied under Nevada conditions. Feeding was the same for each pig; it included four pounds of skim milk and a quarter pound of a mixture of four parts fish meal plus three parts linseed meal daily. Alfalfa pasture was provided in the summer; in the winter three pounds of alfalfa meal were added to the fish and linseed meal mixture. In addition they were given barley according to body weight, varying from 1.2 lb. daily at 30 lb. to 6.2 lb. daily at 200 lb.

In the summer trials using paired crossbred and purebred Duroc pigs of the same weight and sex, the days required for one cwt. gain were  $68 \pm 1.1$  for crossbred and  $73 \pm 0.8$  for purebred pigs. The pounds of concentrates required for one cwt. gain in body weight were  $277 \pm 5.2$  and  $289 \pm 3.4$  respectively. In group trials using eight pigs each in crossbred and purebred groups on pasture, the days required for one cwt. gain in body weight were  $67 \pm 1.0$  and  $71 \pm 2.3$  respectively; the daily gains were  $1.49 \pm 0.014$  and  $1.41 \pm 0.025$  and the pounds of concentrates required for one cwt. gain in body weight were 263 and 268 respectively.

Finally individual and group trials similar to those above were run in the winter. The feeding was the same except that in place of alfalfa pasture, three pounds of alfalfa meal were fed daily. In the trials using paired crossbred and purebred Duroc pigs, the days required for one cwt. gain in body weight were  $70 \pm 1.2$  and  $78 \pm 1.8$  respectively. The pounds of concentrates required for one cwt. gain in body weight were  $287 \pm 5.2$  and  $321 \pm 6.2$  respectively. In the winter group study the days required for one cwt. gain in body weight were  $66 \pm 1.0$  and  $70 \pm 1.4$  and the pounds of concentrates required were 285 and 303 respectively for the crossbred and purebred groups.

It is obvious from these trials that greater gains can be obtained more economically from crossbred Duroc-Poland China pigs than from purebred Duroc pigs. [J. N. W.]

**Breeding for egg production.** L. W. TAYLOR AND I. M. LERNER (1938). *Bull. Calif. Agric. Expt. Sta.* 625.

**T**HE annual production of a hen is a measure of the contributions of all her egg-producing characters. These characters are best studied separately to facilitate selective breeding of a more intense nature.

The age of sexual maturity determines the onset of production. This is easily determined by the age at which the first egg is laid. Climate, nutrition, health, etc. affect this age independent of the heredity of a bird and must be considered in selecting for early maturity. At least two pairs of genes are involved, one of which is sex-linked.

Three kinds of pauses, although admitted by the authors as a term difficult to define, are also important. (1) It is not established as yet that winter pausing is entirely hereditary. The breeder can, however, and should select for breeding only members of those families in which winter pausing is at a minimum or is absent. (2) Broodiness is a character which varies with the breed but is always present. Selection should be made, according to the authors, by the California breeders against broodiness. Two pairs of dominant complimentary genes are involved. Either one alone has no effect, but when both occur in the genotype then the character appears in the phenotype. This fact makes it difficult to eliminate broodiness completely. The importance of broodiness in the annual record of a bird depends both on the number of times the bird becomes broody and on the length of time in each case before the bird resumes laying. (3) Finally, spring and summer pauses occur. It is not known at present whether this character is hereditary, although, such pauses do not occur in the best producers. Selection against it is, therefore, possible for the breeder.

Persistency, or the length of the laying period before the onset of the annual moult, is another important character. The date the hen was hatched seems to have more effect on the age at last egg than on the date at last egg before moulting. Selection for



persistence may, however, be overdone since the moult occurs each fall. If the hen is so persistent as to interfere with or delay moulting, production may not be normal when resumed.

Rate of production is expressed as the percentage of days on which eggs were produced. Gross rate permits confusion of non-pausing birds possessing low intensity with pausing birds possessing high intensity. "Net rate" excludes the days during pauses. Clutch size, or the number of eggs laid on successive days before one day is missed, has been used to indicate the rate of production, but it does not appear to the authors as a basis for successful estimation of this rate.

Viability includes the number of chicks hatched from a given number of eggs, the proportions of these chicks raised to maturity and the mortality of the laying birds. There is good evidence to show that mortality up to the end of the first laying year is partly genetic. The selection of longer-lived strains will result in greater viability. In selecting against diseases the breeder should select breeding birds from those families which are most free of the diseases and not from survivors of less free families.

For measuring flock production the hen-lay average and the average of the records of surviving birds are not preferred to the production index of the Kimber Poultry Breeding Farm. This is a quotient of total eggs laid by a group over the original number of pullets in the group. The prevalence of non-productive or non-viable birds in the flock can best be shown by the Kimber production index since both a high mortality rate and intensive culling reduces the number of pullets after laying has begun. This index is therefore decreased.

A direct correlation exists between body size and egg size, yet the producers of large eggs seldom have a high rate. The authors refer to Hayes as saying that egg size involves one dominant gene for small-eggs and two dominant genes for large eggs. Egg colour is apparently controlled by several pairs of genes, some of them acting cumulatively to produce darker shades, others acting as inhibitors of colour. Shell thickness is probably dependent on a complexity of hereditary factors. Diet, however, is very important in this respect. A considerable number of genes seem to be involved in albumen quality. Hatchability, it is suggested, is controlled by a single gene, although the authors report that some workers question this. Six or more lethal genes are known, however, to cause embryonic death in poultry. [J. N. W.]

**A study of nicking in Jersey cattle.** L. A. JOHNSON, J. W. BARTLETT  
AND LYNN COPELAND (1940). *J. Dairy Sci.* 23, 709.

**NICKING** is generally used in reference to the production of superior progeny from certain matings. Outcross, linebred and inbred matings are used by breeders in the hope of purifying or intensifying good qualities. If one such mating is superior it is said to 'nick'. An unanswered question in this connection, however, is: "Will a bull proved to transmit high producing capacity in one herd be equally successful in another herd where a different family blood line exists?"

In co-operation with the American Jersey Cattle Club a study was made to determine whether or not differences may appear in the producing capacity of various matings. Bulls were selected which had at least two groups of six daughters, of which each group of daughters was out of dams by one sire. All records were converted to a three times a day, 365 day, mature basis. Total fat production was used as a basis of comparison as milk and fat percentage are inherited separately and total fat is a product of the two.

The authors conclude that if nicking is not evident in the mating of a bull with two or three groups of daughters from dams sired by one bull for each group it must not be assumed that nicking will not arise with a different group of daughters. Furthermore, nicking may arise from a group of dams transmitting more producing capacity to their daughters than their own records show.

A bull may nick better with one group of daughters than another and still nick satisfactorily with both. It is also recognised that six cases are too few for as satisfactory a comparison as is possible from a larger number of cases. With a small number of cases, therefore, results may be obtained which would not hold should the number of cases be increased. Environment is also of great importance.

Differences occur between the production of dams and daughters which are not due to the particular mating concerned. It is further concluded, therefore, that nicking may be a term that is freely used in reference to such differences. Its use in such cases is erroneous. Nicking was not shown to be a prevailing factor among Jersey cattle, although its possibility should not be denied. [J. N. W.]

**A successful method of immunization against Newcastle disease of fowls.**

S. GANAPATHY IYER AND N. DOBSON (1940).

*Vet. Rec.* 52, 52, 889.

THE authors have been engaged on the investigation of Newcastle disease, commonly known as Ranikhet disease in India, with particular reference to its control by means of a suitable vaccine. Experiments were undertaken to determine the effect of serial transfers of the virus on the chorioallantoic membrane of the developing chick embryo, using almost the same technique as that described by Burnet (1933 and 1936) and employing the strain of Newcastle virus that was isolated from the 1933 outbreak of the disease in Great Britain and had undergone continued passage through fowls at irregular intervals.

The age of the embryo at inoculation was from 9 to 13 days. The first passage in eggs was effected by the inoculation of a Berkefeld V filtrate of a 1 per cent emulsion in saline of liver and spleen, representing fowl passaged virus. Eggs were opened for examination and collection of the virus on the second or third day after inoculation and reincubation. Death of the embryo was seldom prolonged beyond the second day. For the passage subinoculations, from egg to egg, 0.05 c.c. of a 10 per cent suspension of the infected chorioallantois or the whole embryo, in broth or saline, was used. When eggs, after inoculation with virus, were incubated for over three days, this often resulted in partial or complete loss of virulence of the virus. Consequently the period of incubation after inoculation seems to be an important factor. Further, the character of the lesions is modified by the age of the embryo at the time of inoculation, and the temperature at which the egg is subsequently incubated.

On opening the inoculated egg the chorioallantoic membrane is found to be moist and oedematous with cloudiness arising from cellular infiltration. There are also irregular whitish opaque areas on the membrane indicating ectodermal proliferation followed by, or associated with, vacuolation and necrosis. Small petechial hæmorrhages are fairly numerous and sharply outlined, particularly on the neck and along the sides of the ventral aspects of the body. These hæmorrhages were found on the inside of the wings and the legs, which were highly congested towards the extremities and occasionally on the chorioallantoic membrane. The best lesions were found following the inoculation of eggs on the 9th or 10th day of incubation.

The work of Burnet and Ferry (1934) indicated that there was no modification in the virulence of the Newcastle virus as a result of twelve passages through eggs. But the present authors' observations showed the necessity for more than twelve passages before the fowl virus could be attenuated. They found that the virus after cultivation through eggs for 19 passages remained fully virulent for adult fowls but the fowl subinoculations carried out at the 33rd and the subsequent passages upto the 56th revealed the fact that the strain of virus had undergone a change, and ceased to cause fatal results in them and had developed immunizing properties. About two weeks after the injection of healthy fowls with the egg-passage virus, without any treatment with chemicals (to cause attenuation), they developed immunity which was solid enough to withstand subcutaneous injection of 1 c.c. of fowl-passage virus containing at least 100 million fowl minimal lethal doses and constituting a much more severe exposure than would occur under field conditions.

The experiments were repeated and periodic infectivity tests were made and the results showed that in this line of the same strain of virus even after 14 passages through eggs, the virus was sufficiently attenuated to be used as a vaccine with safety and reliability. The authors are of opinion that this variation in the number of passages required for proper attenuation may be due to such factors as the individual susceptibility or age of the embryo at the time of inoculation, the duration of re-incubation of eggs following inoculation, the occasional necessity for storage of the virus in the refrigerator between passages, owing to the shortage of suitable eggs for inoculation, and the individual susceptibility of the test fowl.

Although the egg-passaged virus became attenuated for fowls after a certain number of passages yet it continued to be lethal for the embryos in 48 hours even at a dilution of  $0.05 \times 10^{-6}$  g. A short experiment carried out at the 41st passage showed that the egg-cultivated virus after having undergone attenuation did not return to its original virulence following two passages through fowls, suggesting its safety for using as an immunizing agent under field conditions. Experiments to determine the effect of drying and storage of the egg-cultivated virus and further work in regard to its propagation through fowls while retaining its safety and efficiency as a vaccine are in progress.

Owing to the marked susceptibility of young chicks the vaccination of fowls under eight weeks of age is not recommended.

[ R. L. K.]





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## ORIGINAL ARTICLES

### STUDY OF WOOL QUALITY AS THE BASIS OF INDIVIDUAL SELECTION IN BREEDING OF DECCAN SHEEP\*

BY

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(Received for publication on 21 October 1940)

(With Plates XIV—XVII and 8 text-figures)

#### INTRODUCTION

THE Deccan sheep lacks the purity of type which selection and breeding to a definite purpose can stamp in a breed. There is no uniformity in conformation or in wool bearing character in these sheep. The wool is in every phase of evolution from its wild state. It appears in all colours and conditions. In a general way the shepherd selects a black ram and thus the majority of the Deccan sheep are black-coated with all the variations of brown, blue and roan mixtures. The black colour of the fleece is not permanent. After a couple of shearings, the black takes a rusty tinge and after the fourth shearing it degenerates into greys and roans. The farm had to settle first the policy of selection of sheep for uniformity of colour and the only method of doing this was by stocking the farm with a white flock of Deccan sheep. Yet an all-white sheep could not be selected. It lacks stamina and no shepherd in the Deccan retains such an animal after the period of weaning. A black or mottled colour on the face along with black hoofs must be bred in the flock to retain stamina (Plate XIV, fig. 1).

At the time when these sheep were first purchased, there was no idea of the quality of the fleece or the types of fibres existing on these animals. The majority of the Deccan sheep cannot be said to carry 'a fleece' in the right sense of the term. A clip from these sheep is a mixture of hair and wool, the major portion comprising of the former (Plate XVI, fig. 2). The sheep on the farm are not shorn by the shepherd, but by the Manager and his assistants. As a result, it was found that certain sheep carried a better coat than the rest, and the study of wool quality as the bases of selection had its origin in this constant handling of the seasonal clips. For rough classification these sheep were classed into three grades.

\* The experimental work reported in this article was carried out at the Sheep Breeding Farm, Poona, during the year 1939-40 under a grant from the I. C. A. R.

In sheep which have been bred to carry a real fleece meaning only wool fibres, a breeder has a chance to pick up fleeces of exemplary quality for his stud breeding. But it was found that the coat of the Deccan sheep has a mixture of fibres varying from a fine wool to a rough stiff hair, with all the intermediate types. A detailed study of these fibres was made and they were classed into (1) fine wool, (2) strong wool, (3) heterotypes, (4) hair, and (5) kemp. Thus to get out of this maze of variations from sheep to sheep, a standard had to be fixed for study and selection. It was decided to segregate sheep which carried wool fibres only in their fleeces or in other words those animals which bore no hair in the fleece-bearing part of the body. This phrase, 'fleece-bearing part of the body', has been purposely included here because the Deccan sheep has no wool on the head, chest, belly, legs and on the lower part of the neck. These parts are covered with stiff hair. A shepherd in the Deccan who has very little idea of quality of wool when scraping these hairy portions at the time of shearing, mixes them with the rest of the clip, and thus reduces its quality to almost a waste. Leaving aside this negligence in shearing, the selection of Deccan sheep will have primarily to be based on the percentage of hair or in other words medullated fibres in their fleeces. When once the standard of quality is fixed according to the medullation percentage, the rest of the attributes of wool, viz. length, fineness, waves or crimps and percentage of kemp will give the fleece a definite place in selective breeding. This study is primarily meant for selection of stud flocks. Thus the existing farm flock is classed into :—

*Grade A.*—Sheep carrying fleeces containing no hair.

*Grade B.*—Sheep carrying fleeces containing long hairy fibres which have the characteristics of growing without the seasonal break.

*Grade C.*—Sheep carrying fleeces containing short hairy fibres which are seasonally shed.

(Plate XV)

#### IMPORTANCE OF WOOL CLASSIFICATION

In the following pages the method of wool study of the above 'A' grade sheep is given. Before entering into the details of this subject, it will not be out of place to state the importance of the above method of sheep classing. The Deccan wool is mostly used for the production of rough country blankets. Except this, the wool has very little economic value. The spinning limit of this wool is from 2 to 5 counts (woollen). Exceptional fine blankets are prepared from yarn of 10 counts. The 'A' grade mentioned above can easily be spun up to 30 counts and the 'B' grade into 15 counts. The 'A' grade wool will take dye better than the 'B' and 'C' grades which absorb dyes indifferently. This is from the utility point of view, but it is the study of wool analysis of individual sheep that is going to influence the future sheep-breeding work of this part of the country. Although the Deccan sheep are at present in an undeveloped stage, this carries with them the advantage of adaptability. One will have to take advantage of this character in evolving a new breed either by selection or by crossing. Examination of the fleeces in detail has shown that a sheep carrying wool fibres can be selected out of this heterogenous mass. It follows then that detailed record of such sheep should be maintained. This requires the study of a large number of sheep.

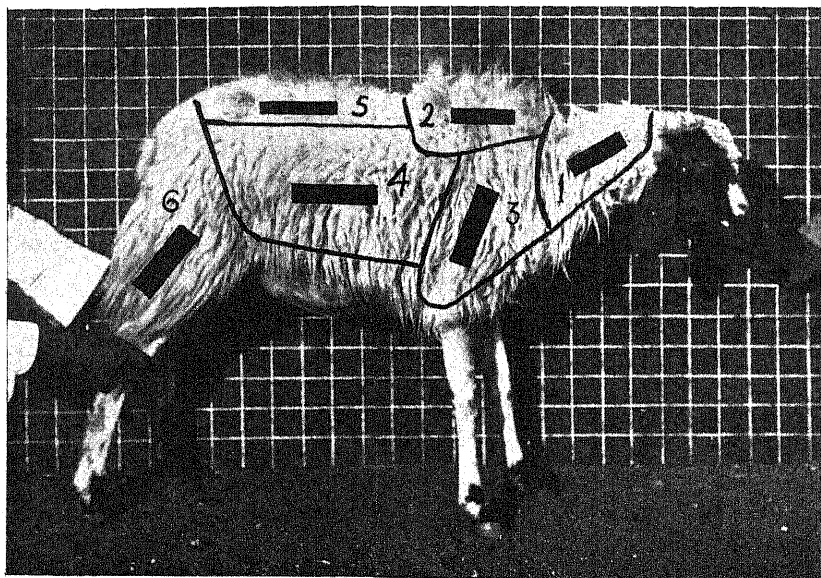
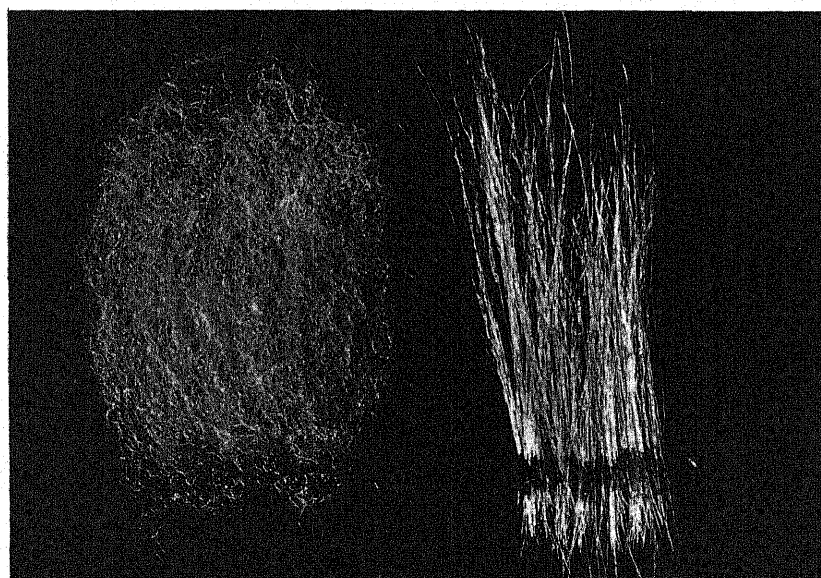


FIG. 1. Regional demarkations and places where samples are taken

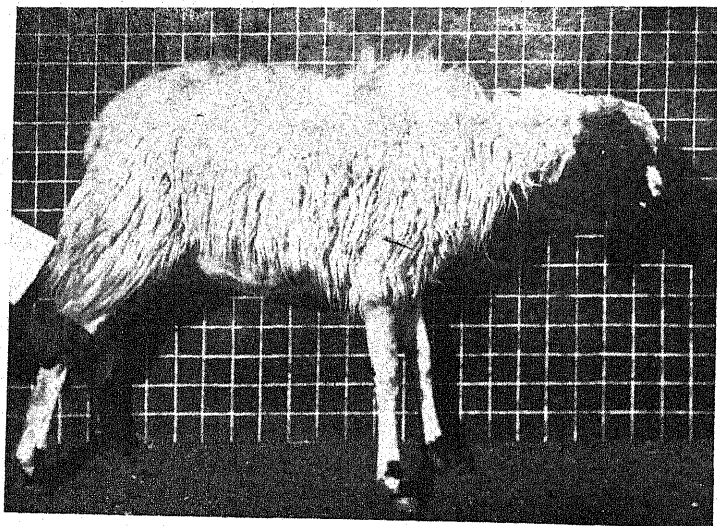


FINE

MED—FIBRES

FIG. 2. Types of fibres in the fleeces of ewes Nos. 7, 69 & 5





Types of Deccan sheep segregated on the basis of wool quality

FIG. 1. Grade 'A'—D ♀ 4

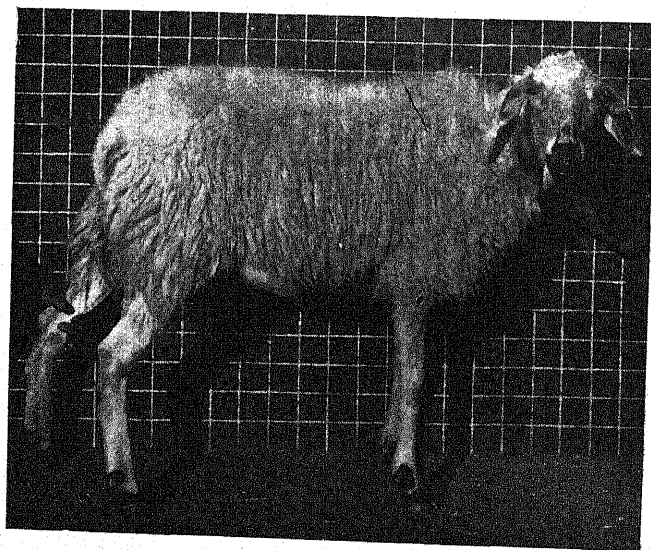


FIG. 2. Grade 'B'—D ♀ 41

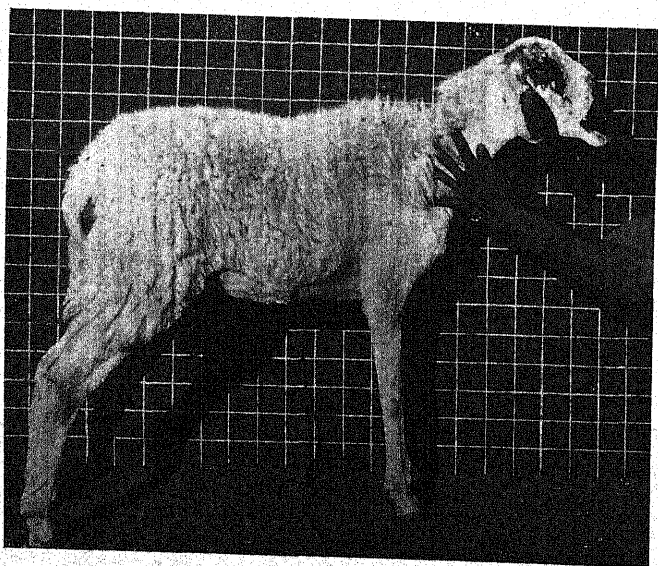


FIG. 3. Grade 'C'—D ♀ 19



From the existing farm flock ten ewes of the 'A' grade type were selected for the following fibre study. A total population of 18,000 fibres was studied from the fleeces of these sheep. The Deccan sheep are shorn twice a year and it is the January 1940 clip that has been handled for the following details.

#### WOOL ANALYSIS OF THE 'A' GRADE DECCAN SHEEP

##### *Sampling and zoning*

The first important item in the study of a fleece is correct sampling. A sample should be representative of the whole fleece covering every part of the body which contributes to the total yield. It is easy to work the minute details of measurements and then record them in terms of statistical methods in a laboratory, but the field sampling on which the laboratory work has to be based must be carried out with great care and by persons who know sheep and wool. The wool-carrying portion of this type of Deccan sheep was first demarked into six zones (Plate XIV, fig. 1). These are:—(1) neck (dorsal), (2) wither, (3) shoulder, (4) side, (5) back, and (6) britch. Samples were taken from both sides of the body on the corresponding regions. The area covered was not less than eight square inches and more in the case of back and the side wools, as these regions are bigger in dimensions. The regions from which these samples were taken are shown in Plate XIV, fig. 1. Further a representative sample was prepared for each region by the halving method. Every fibre from this composite sample was analysed into different types of fibres.

##### *Method*

As stated above a composite sample was prepared for each zone. It was steeped overnight in water and washed in three changes of distilled water and after thorough drying, cleaned of its grease by washing it in petrol and finally in warm benzene. Each fibre was separately examined and classed. These component parts of the samples were weighed in a constant humidity chamber (by courtesy of Dr Nazir Ahmad, at the Cotton Technological Laboratory, Matunga, Bombay), on a Torsion balance at 70 per cent humidity and from this the percentage of types of fibres on different zones and subsequently for each fleece was calculated.

In the absence of a microprojector of the type of Zeiss Lanometer or Wira projector the measurement of diameter of fibres was carried out with the aid of a microscope. However, the work was hastened by the use of the Maturity Slide suggested by Dr Nazir Ahmad. A hundred fibres from each class were picked by tabbing (every fifth fibre being taken) and cut in three places. These were mounted under cedar wood oil on the maturity slide. A hundred readings for every type of fibre for each zone were recorded. This makes approximately 600 readings for each type of fibre per fleece. Thus not less than 5,500 readings for diameter measurements were recorded for each type of fibre existing in 'A' grade Deccan sheep.

Length measurements for each group of fibres were carried out on a velvet board by stretching the fibre gently against a steel rule until the

waves or the crimps just disappeared. The method of tabbing was also followed here. The same number mentioned above for fineness determination was recorded for length measurement.

The fibres termed 'heterotypes' in this work are the partially medullated fibres in the fleeces. The weights of these denote the percentage of medullated fibres in that sample, and not medullation percentage which actually shows the total medullation value of a particular sample. The medullation percentage can correctly be measured by the Medullometer devised by McMahon at the Massy Agricultural College, New Zealand. The study was restricted to recording the percentage of medullated fibres.

These fibres after being classed by hand were tested both by the Elphic Benzol method and by keeping a careful scrutiny when spread for measurement of diameter under the microscope.

#### CLASSIFICATION OF SUBJECTS

1. Study of types of fibres.
2. Study of the total population of fibres irrespective of types.
3. Determination of minimum number of tests for obtaining a reliable mean for measurements of diameter and length for each type of fibre.
4. The quality of wool on different zones.
5. Analysis of variance.
6. Method of recording individual and progeny performances.
7. Miscellaneous notes in the study of wool quality.

##### *1. Study of types of fibres*

There are four types of fibres in the fleece of 'A' grade Deccan sheep, viz. (1) fine fibres, (2) strong fibres, (3) heterotypes and (4) kemp.

*Fine fibres.*—This term has been used because these fibres can be classed with the fine wools like those of the Merino. These are extremely pliable and give a downy feel. They are covered with fine serrated scales and are distinctly crimped with approximately ten crimps per inch, but the crimps are uneven. An increase of the fine fibres in the fleece will give it the felting quality which the average Deccani wool lacks. The study of monthly growth of fleece has shown that this fibre can grow continuously. It has a more even staple than the other fibres. Length measurements were taken from region to region. These results are consolidated in Table I according to regions.

The average length based on 6,000 readings is 2.28 in. for a six-monthly growth, the standard deviation being 0.483 with the coefficient of variation of

TABLE I

*Table of constants for length measurements*

Samples	Fine fibres				Strong fibres				Heterotypes				Kemps			
	Mean	S. D.	C. V.	No.	Mean	S. D.	C. V.	No.	Mean	S. D.	C. V.	No.	Mean	S. D.	C. V.	No.
Neck	2.41	0.0156	20.42	1,000	3.45	0.0228	20.05	1,000	2.84	0.0209	22.04	898	1.21	0.0311	25.05	230
Wither	2.56	0.0155	19.18	1,000	3.57	0.0211	18.63	1,000	3.05	0.0214	21.24	954	1.95	0.0287	24.20	270
Shoulder	2.11	0.0136	20.38	1,000	2.81	0.0228	21.42	700	2.48	0.0222	23.07	660	1.67	0.0237	30.54	450
Side	2.29	0.0113	15.92	999	3.10	0.0237	20.38	730	2.55	0.0237	22.36	630	1.69	0.0254	25.28	284
Back	2.28	0.0141	19.56	1,000	2.46	0.0153	18.59	890	2.15	0.0155	19.13	700	1.74	0.0233	23.60	340
Britch	2.00	0.0137	21.65	1,000	2.74	0.0234	25.62	900	2.51	0.0224	23.58	700	1.84	0.0327	30.65	305

21.18 per cent. Fig. 1 shows the characteristic graph of these fibres very clearly.

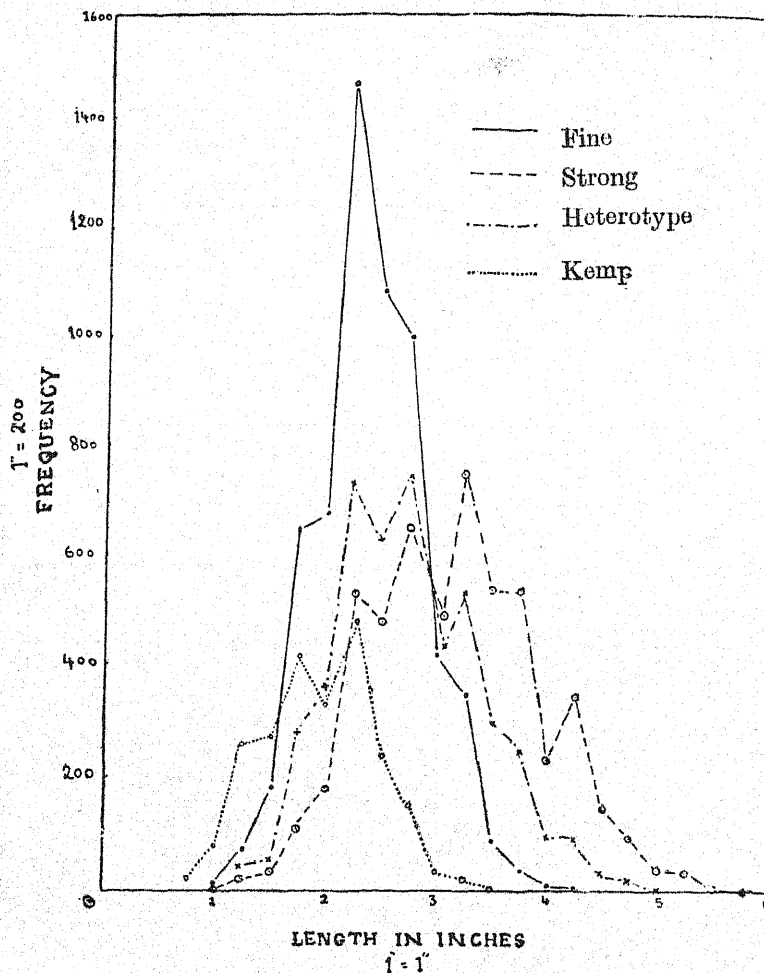


Fig. 1. Length frequency

The variation in length from region to region is from 2.0-2.56 in. The maximum length is seen in the wither wool and minimum in that of britch (Fig. 5).

The mean diameter of these fibres for 6,000 readings is  $25.30\mu$ , the standard deviation comes to  $5.32\mu$  with a coefficient of variation of 21.00 per cent. The table of constants for diameter measurements is given in Table II. The readings for diameter of this fibre will be seen to vary from  $24.90$ - $26.77\mu$ . This shows that this fibre is extremely even in its diameter. It can be definitely said that in the Deccan wool this is the most even fibre both for length and diameter (Fig. 2).



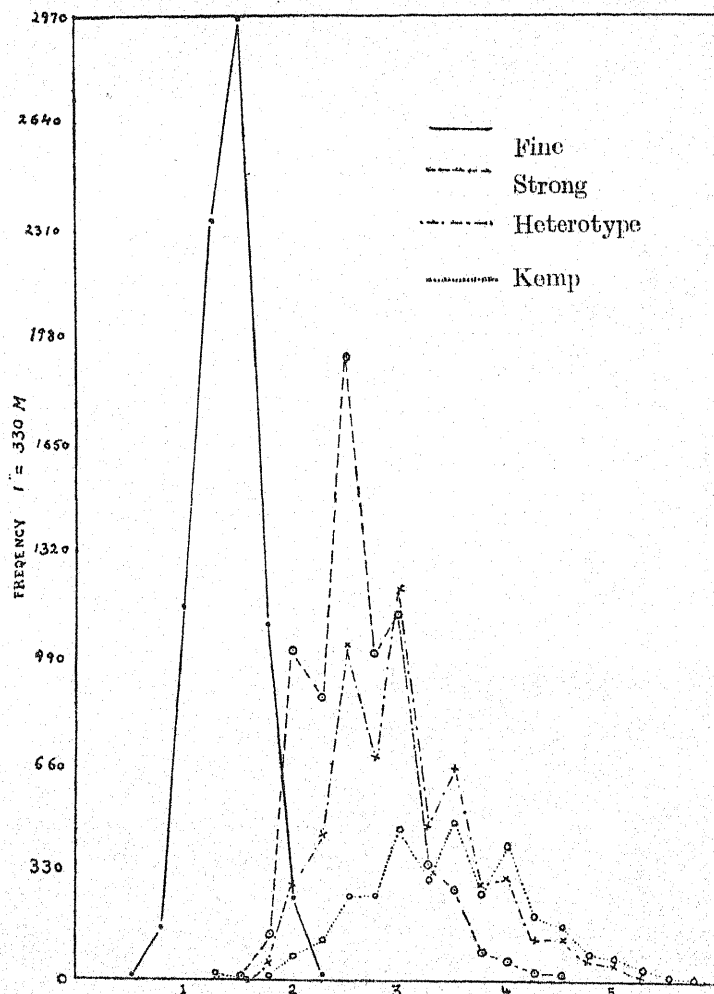


Fig. 2. Diameter frequency

*Strong fibres.*—The term 'strong' is used in wool-classing whenever the fibres have good textile strength and in a class of fleeces a strong wool type generally denotes a fleece having fibres of comparatively larger diameter. This adjective has been purposely affixed here because these are the dominant fibres in the 'A' grade fleece of the Deccan sheep. These fibres give the characteristic strength and quality to the wool as a class by itself. As stated above the fine fibres render a felting quality while the strong wool gives the 'character' and strength to the fleece of these sheep. It is pliable and wavy with approximately two waves per inch.



TABLE II  
*Table of constants for diameter measurements*

Samples	Fine fibres				Strong fibres				Heterotypes				Kemps			
	Mean	S. D.	C. V.	No.	Mean	S. D.	C. V.	No.	Mean	S. D.	C. V.	No.	Mean	S. D.	C. V.	No.
Neck	25.415	0.153	19.0	1,000	47.25	0.2413	16.14	1,000	56.16	0.4224	22.08	862	68.28	0.8011	20.32	306
Wither	24.90	0.1635	21.02	1,000	51.31	0.2796	16.84	1,000	59.44	0.4015	20.29	902	65.86	0.6692	19.90	327
Shoulder	25.15	0.1655	20.0	1,000	51.58	0.3605	18.49	690	51.04	0.4541	22.44	657	59.47	0.5384	22.18	600
Side	25.63	0.1638	19.44	1,000	47.20	0.3370	19.16	720	57.10	0.5346	23.17	611	68.42	0.6692	20.21	403
Back	26.08	0.168	20.34	998	50.79	0.3640	20.86	850	61.96	0.5332	22.19	665	70.29	0.6861	20.57	443
Britch	26.77	0.1746	20.62	1,000	49.63	0.2765	17.71	899	59.76	0.4677	20.60	693	68.27	0.6455	18.95	402

The length measurement of these fibres was carried for every zone. The mean length as calculated from 5,200 readings comes to 3.05 in. with a standard deviation of 0.765 and a coefficient of variation of 25.00 per cent. The length is highest in the wither wool—3.57 in., and least in the back wool—2.46 in. (Table I). It more or less follows the level of the fine fibres. The curve as shown in Fig. 1 shows a slight deviation. This is due to uneven fibre length of the back wool.

The diameter readings for these fibres totalled 5,100 for ten fleeces. The mean diameter works to  $49.66\mu$ , with a standard deviation of 9.20 and a coefficient of variation of 18.52 per cent. The diameter was also measured for six zones of the ten fleeces. It must be noted here that three ewes, Nos. 7, 69 and 5 under experiment, had no fibre of this type in their shoulder and side wools. Ewe No. 69 showed the existence of a few of these fibres in the side region. This inconsistency is also seen in the back and the britch wools of these ewes. Statistically it can definitely be laid down that these three ewes out of the ten are a class by themselves. Yet in general, this conclusion should not be a final one unless a large number of Deccan sheep are studied. The diameter readings show that the fibre varies from  $47.20\mu$  in the side wool to  $51.58\mu$  in the shoulder wool (Table II).

*Heterotypes.*—These are coarse medullated fibres with different phases of medullation. In a single fibre both medullation and non-medullation exist. The fibre presents an appearance of irregularity in waves and lustre. The medullated portion shines like a glass and is stiffer than the non-medullated region, which gives it sudden twists and a change of axis with irregular thickening at the junction of the medullated and non-medullated portions. In a yarn the medullated portion of these fibres comes out of the general pile and gives a rough texture to the cloth. Due to the indifferent absorption of the dye-stuff by these fibres, the fabric has an uneven appearance. They nearly have the same length as the strong wool. Length measurement of 4,500 fibres was recorded. The mean length comes to 2.63 in. with a standard deviation of 0.657 and a coefficient of variation of 25.00 per cent. The length frequency curve of this fibre (Fig. 1) is of the same type as that of the strong wool. The lengths of these fibres were worked out into frequency groups. The maximum length of these fibres is in the wither wool; next stands the neck, the minimum being in the back wool (Table I).

A population of 4,400 was measured for the determination of the diameter. The mean diameter comes to  $57.89\mu$  with a standard deviation of 12.82 and a coefficient of variation of 22.18 per cent. The frequency diameter curve (Fig. 2) shows that the diameter is more variable than the other fibres. The diameter measurements from region to region were worked out. The table of constants for diameter measurements (Table II) shows a variation from  $51.94\mu$  in the shoulder wool to  $61.96\mu$  in the back wool.

*Kemp.*—This is a very important fibre in so far as its existence is a disqualification in a fleece. The kemp is generally described as the coarsest of the fibres. It is stiff, short and is fully medullated. It grows straight from the skin end and then develops into a spiral and ends in a very thin pointed non-medullated structure. The typical kemp existing in an average Deccan sheep is stiff, opaque and chalky fibres (Plate XVI, fig. 2). At the base

it starts with a thickness of  $52-61\mu$  and in its straight growth broadens up to  $80-104\mu$ . Then it takes a spiral growth having a breadth of  $90-114\mu$  and at the tip it turns suddenly into a non-medullated structure of a fineness of  $7\mu$ . But in 'A' grade sheep kemp takes a less severe structure. It starts at the proximal end with a diameter of  $49-76\mu$  and continues with about the same breadth for three-fourths of the length and begins its pointed tip which ends in  $5.2\mu$ . The various types can very well be compared by referring to Plate XVI. In these sheep they have lost the chalky appearance and have developed a flinty translucency. The measurements of 2,300 kemp for length are worked out in frequencies. The mean length works to 1.82 in. with a standard deviation of 0.50 and a coefficient of variation of 27.52 per cent.

The diameter measurements although not a very important factor, were recorded for 2,300 fibres to understand what type of kemp exists in these sheep. The mean diameter comes to  $66.30\mu$  with a standard deviation of 14.14 and a coefficient of variation of 28.30 per cent. The curve in Fig. 2 will show the variation in the population of these kemp.

*Medullated fibres.*—Peculiar fibres of the type of a degenerate hair were seen in three ewes Nos. 7, 69 and 5. These fibres grow straight like hair, have a shining appearance with slight medullation. The epithelial scales are broad, rounded and are more like those of hair than those of wool. They seem to be some middle stage between strong wool and hair. They are extremely even in diameter and lack the waviness and pliability of the true strong wool described above.

In these ewes, it was found that the fine wool and these fibres dominated in the fleeces (Table X). It is not possible to lay down whether such sheep are a type by themselves unless a large flock of 'A' grade is studied.

## 2. Study of the total population of fibres irrespective of types

This has been dealt with here finally on the fibre study because each of the types mentioned above is a separate entity. The reason for classing and treating each type of fibre independently for length and diameter measurements is very well illustrated by Figs. 3 and 4, one showing the frequencies for length and the other those for diameter. The heterogeneity of the population is evident from these graphs.

## 3. Determination of minimum number of tests for obtaining a reliable mean for measurements of diameter and length for each type of fibre

In every research which requires measurements of a population for a study of a type or types, it is now a known fact that the most essential work needed

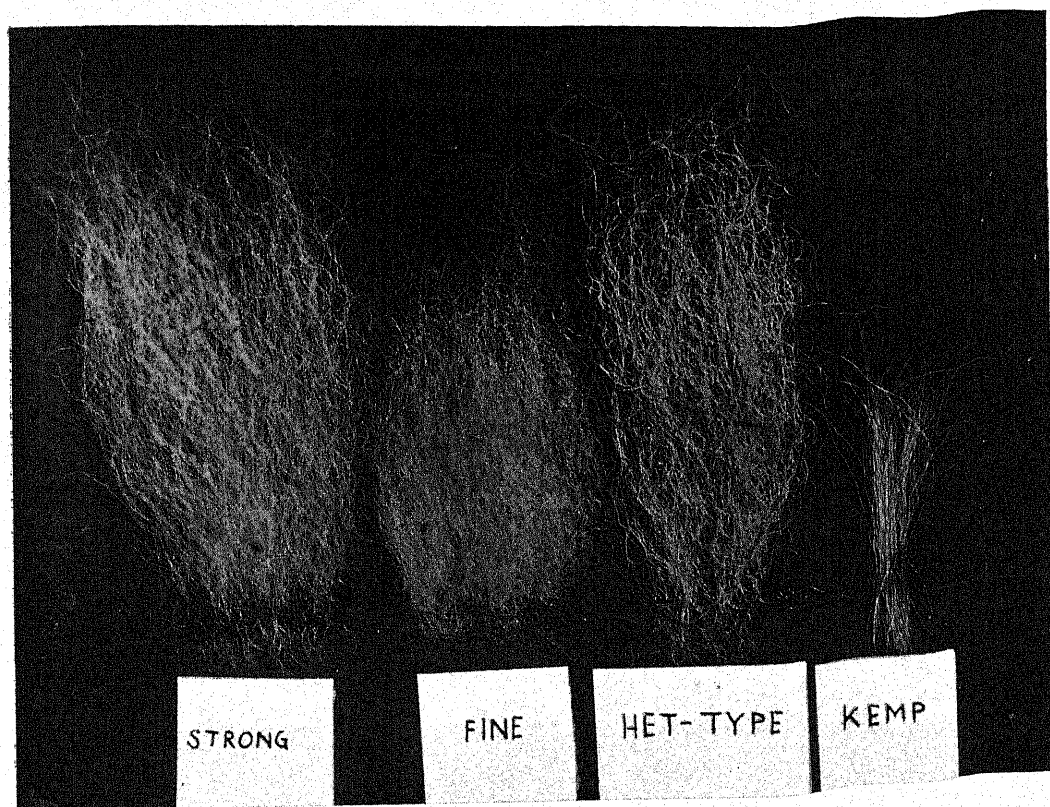


FIG. 1. Types of fibres in 'A' grade Deccan sheep. The hairy fibres which go to make the bulk of the 'B & C' grade are absent in these sheep

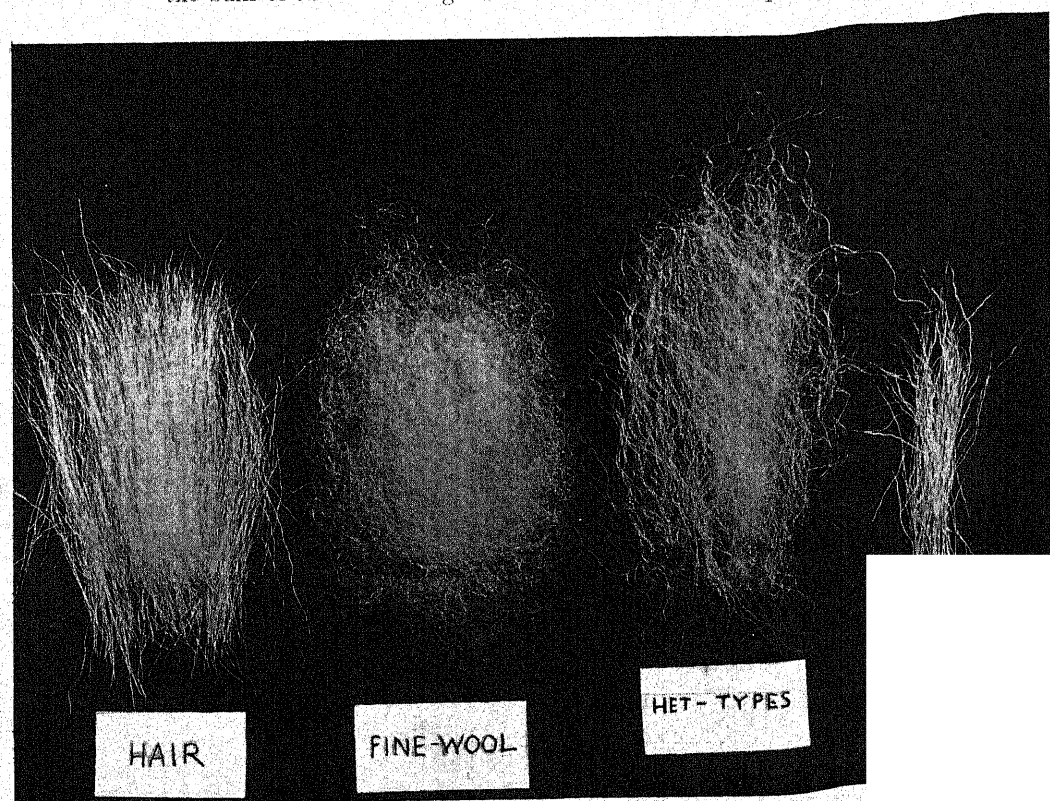
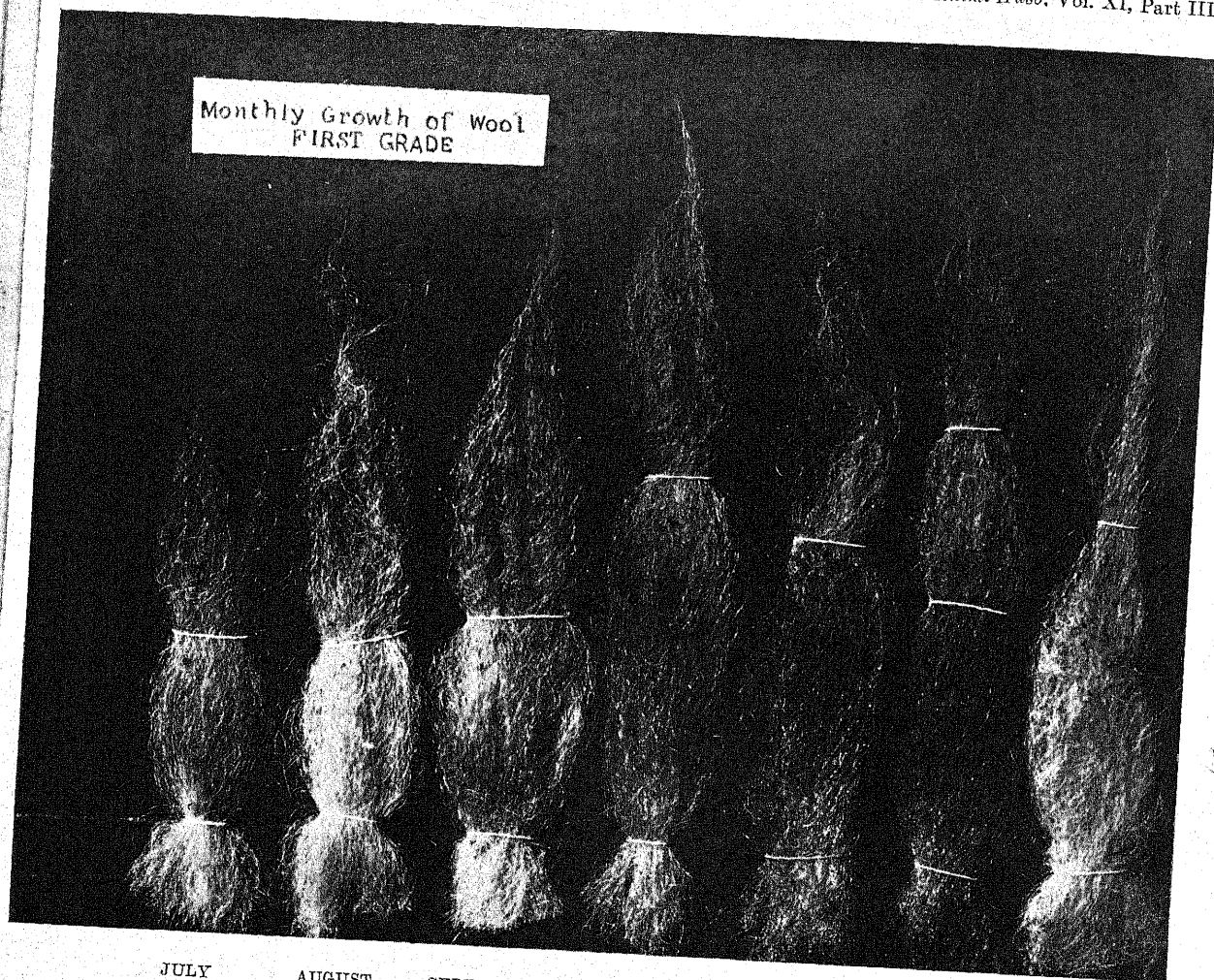
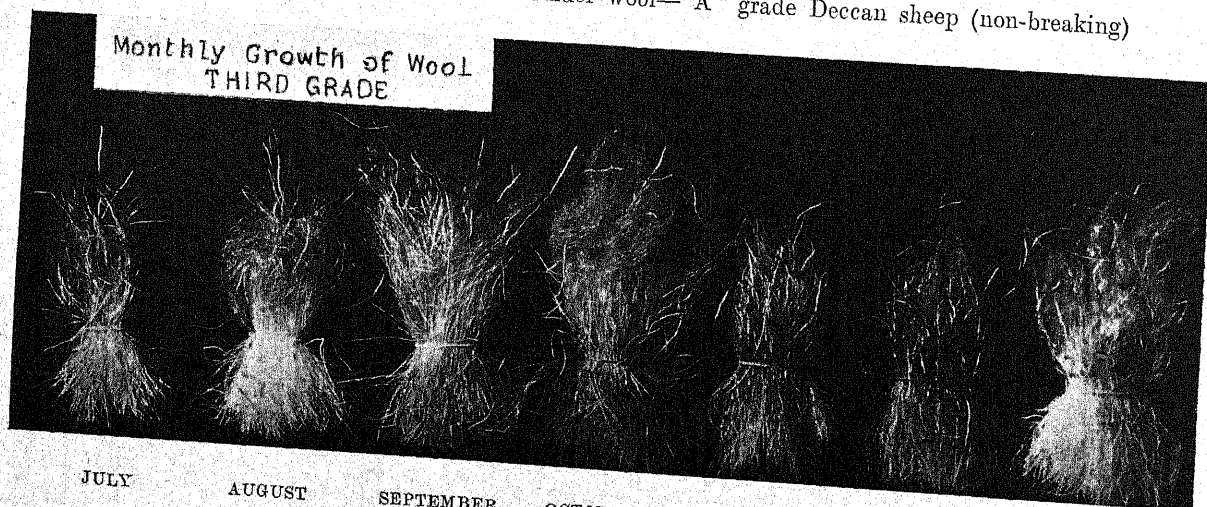


FIG. 2. Types of fibres in 'C' grade Deccan sheep for comparison





JULY AUGUST SEPTEMBER OCTOBER NOVEMBER DECEMBER JANUARY  
FIG. 1. Monthly samples of the shoulder wool—'A' grade Deccan sheep (non-breaking)



JULY AUGUST SEPTEMBER OCTOBER NOVEMBER DECEMBER JANUARY  
FIG. 2. Monthly samples of shoulder wool—'C' grade Deccan sheep (breaking type)



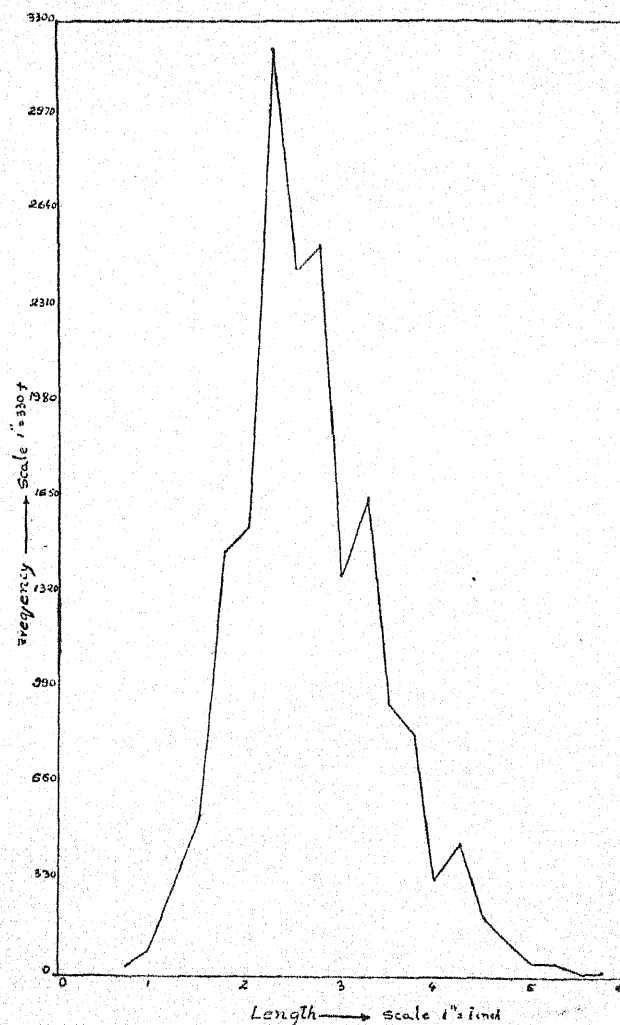


Fig. 3. Length frequency of the total population (irrespective of types of fibres).

at the outset is to calculate the minimum number of counts which should be recorded in obtaining a reliable mean for that population. This is ordinarily calculated after recording readings of a fairly large number and finding out the standard deviations along with the coefficients of variation. The minimum

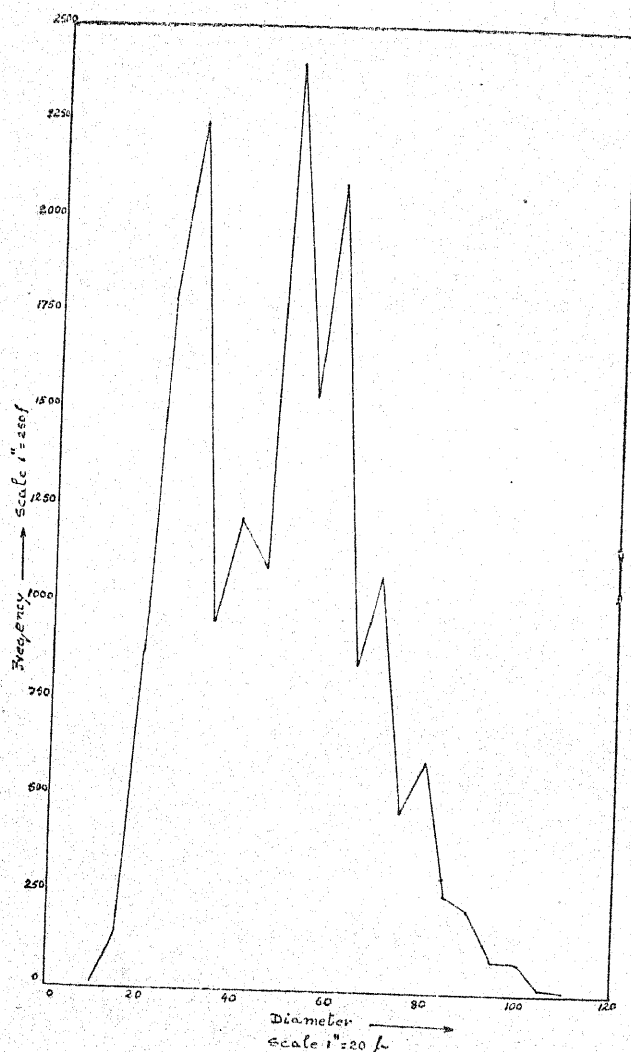


Fig. 4. Diameter frequency of the total population (irrespective of types of fibres).

number of tests is then calculated for each type according to the following formula :—

$$N = \frac{m^2 \sigma^2}{x^2} \text{ where --}$$

$N$  is the number of tests ;

$x$  is the desired accuracy expressed as percentage of mean ;

$\sigma$  is the percentage standard deviation (coefficient of variation) ; and

$m$  is the factor for obtaining specific odds.

Thus for odds of 19 : 1  $m = 1.96$  and for 99 : 1 odds its value is 2.58.

In the present study a hundred readings for diameter and length are carried out for each type of fibre in every zone. Thus the total number of fine fibres measured amounts to 6,000, and that of the strong fibres to 5,200 and lastly 4,500 for the heterotypes. The coefficients of variation for each type of fibres are recorded in Tables I and II.

*Minimum number of tests.*—For both the length and diameter measurements over a population of 6,000, the coefficient of variation comes to 20.00 per cent in the case of fine fibres, it comes to 25.00 per cent for length measurement and 20.00 per cent for that of diameter in strong fibres. In heterotypes it averages to 23.00 per cent for both the fineness and length measurements. With these values for  $\sigma$  the above formula has been applied and the values of  $N$  for different percentages of accuracy for  $P=0.05$  and for  $P=0.01$  are recorded in Tables III-VI.

TABLE III

*Number of tests of length and fineness for specified accuracy (fine fibres)*

Value of $X$ for $P = 0.05$	No. of tests $N$	Value of $X$ for $P = 0.01$	No. of tests $N$
0.5	6146	0.5	10640
1.0	1537	1.0	2662
1.5	682.9	1.5	1183
2.0	384.2	2.0	665.6
2.5	246.0	2.5	426.0
3.0	170.8	3.0	295.8
3.5	125.4	3.5	217.3
4.0	96.01	4.0	166.3
4.5	75.90	4.5	131.4
5.0	61.46	5.0	106.4
5.5	50.79	5.5	87.98

TABLE IV  
*Number of tests of length for specified accuracy (strong fibres)*

Value of $X$ for $P=0.05$	No. of tests $N$	Value of $X$ for $P=0.01$	No. of tests $N$
0.5	9603	0.5	16630
1.0	2401	1.0	4159
1.5	1067	1.5	1848
2.0	600.3	2.0	1040
2.5	384.2	2.5	665.6
3.0	266.8	3.0	462.2
3.5	196.0	3.5	339.4
4.0	150.1	4.0	259.9
4.5	118.5	4.5	205.4
5.0	96.03	5.0	166.3
5.5	79.36	5.5	137.5

TABLE V  
*Number of tests of fineness for specified accuracy (strong fibres)*

Value of $X$ for $P=0.05$	No. of tests $N$	Value of $X$ for $P=0.01$	No. of tests $N$
0.5	6145	0.5	10640
1.0	1537	1.0	2662
1.5	682.9	1.5	1183
2.0	384.2	2.0	665.6
2.5	246.0	2.5	426.0
3.0	170.8	3.0	295.8
3.5	125.4	3.5	217.3
4.0	96.0	4.0	166.3
4.5	75.90	4.5	131.4
5.0	61.46	5.0	106.4
5.5	50.79	5.5	87.98

TABLE VI

*Number of tests of length and fineness for specified accuracy (heterotypes)*

Value of $X$ for $P=0.05$	No. of tests $N$	Value of $X$ for $P=0.01$	No. of tests $N$
0.5	8128	0.5	14080
1.0	2032	1.0	3521
1.5	903.3	1.5	1564
2.0	508.2	2.0	880.2
2.5	325.2	2.5	563.3
3.0	225.8	3.0	391.2
3.5	165.9	3.5	287.4
4.0	126.8	4.0	220.0
4.5	100.3	4.5	173.9
5.0	81.28	5.0	140.8
5.5	67.17	5.5	116.3

From the results it is evident that in the case of fine fibres the present experiment is correct to 4 per cent accuracy on either side of the mean for  $P=0.05$ . The accuracy works out to 5 per cent in the strong fibres for length and 4 per cent fineness for  $P=0.05$ . In the case of the heterotype the experiment is correct to 4.5 per cent accuracy for  $P=0.05$  for fineness and length measurement as well. Hence the fleece analysis carried out in the present study can be said to give reliable accuracy of 5 per cent for  $P=0.05$  in calculating the mean for diameter and length measurements by taking a hundred tests for each type of fibre.

*Is there a short-cut for quick analysis of the fleeces of Deccan sheep?*—One may think that the method followed above in the classification of fibres by recording the detailed measurements is going to be a tedious process, when a large number of sheep are to be studied. The McMahon 'Medullometer' (1934) when used by trained persons will be able to deal with thousands of samples in a short time. The quality of each fleece can then be measured irrespective of classification of fibres by preparing a composite sample from all the zones. From these again a representative sample can be prepared and measured for all the attributes of wool which go to form the quality. Supposing the 'A' grade sheep are a fixed type, it will then be seen that in the case of diameter measurements, 390 readings and 205 for those of length will give an accuracy of 5 per cent on either side of the mean for  $P=0.05$  to obtain reliable means.



4. *Quality of wool on different zones*

In improvement of sheep for wool bearing character uniformity of quality is of the highest importance. As explained previously, there is a great variation in quality from fleece to fleece in the Deccan sheep. The present study is of the 'A' grade Deccan sheep selected to carry a fleece having no hairy fibre. In the breeding of such animals one must go a step further and find out the variation of wool quality on different parts of the body. The drive in selection should be towards a sheep having uniform superior wool all throughout the fleece. This can only be achieved by regional studies and recording these observations for each generation for selecting the stud stock. The principal attributes of wool which should be given importance in judging the uniformity for the Deccan fleeces at this stage should be medullation percentage, length, fineness, and percentage of kemp. Out of these, the medullation percentage and uniformity in length are of primary importance. The fineness of fibres and the percentage of kemp are to guide the final selection. For the ten ewes under study the details of the types of fibres are already given. At the same time proportions of each of these on different zones has been completely worked out. These results are consolidated in Table VII.

TABLE VII

*Percentage of different types of fibres on different regions*

Regions	Fine fibres	Strong fibres	Medullated fibres	Remarks
Neck	24·32	56·49	19·17 (3·56)	The figures in brackets show the percentage of kemp.
Wither	33·52	44·85	22·35 (4·59)	
Shoulder	22·56	62·97	12·37 (4·25)	
Side	27·59	40·00	32·38 (12·52)	
Back	36·57	31·75	31·66 (9·26)	
Britch	17·41	42·12	40·45 (8·98)	

The total medullation percentage could not be worked out due to want of apparatus. The total weight of heterotypes has been taken to calculate the percentage of medullated fibres. From the point of medullation shoulder wool stands first in quality followed by neck and wither wools. On the whole in fleece classing of the Deccan sheep at the present stage of breeding, these

three regions can safely be placed together as one quality. The side and the back wools are almost alike except that the side wool has a greater percentage of kemp than the back wool. The kemp is said to be an heritable character and the students of wool and sheep advise that these should be separately estimated. However, with the quality of wool which is under study, it is advisable to take the total medullation as the primary factor in judging sheep and then correct the results by other attributes in the following order of their importance: (1) length, (2) fineness, (3) percentage of kemp, (4) crimps (or waves), and (5) density.

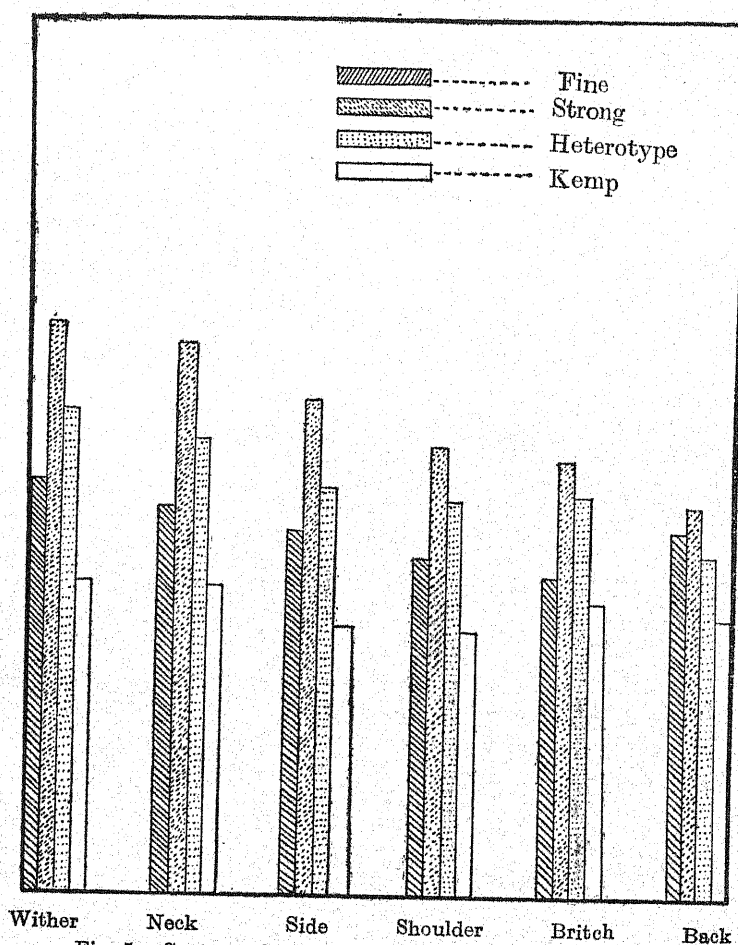


Fig. 5. Contour of the fleece of the 'A' grade Deccan sheep.

The histogram (Fig. 5) given above will show the general stand of the coat of the Deccan sheep from region to region. It is seen that the wither wool has the longest fibre followed by neck and side wools. The back wool has the least length. This contour of the fleece is a common factor in the majority of the Deccan sheep. The most defective part of the fleece is the

back wool. In the selection of animals this defect will have to be eliminated gradually. The britch wool in any breed of sheep is classed as the lowest quality. This is also true of this breed and the figures of medullation given in Table VII are self-evident.

The fineness of wool on a zone depends on the proportion of types of fibres. The percentage of fine or coarser fibre will change the mean diameter of the total population. Hence the proportions of the types and their quality are of more importance than the mean diameter for all types of fibres on a zone or in a fleece.

On the whole, it can be laid down that the fleece of the Deccan sheep can be classed into four groups at the shearing yard :—

1. Neck, wither and shoulder wools.
2. Side and back wools.
3. Britch wool.
4. Hairy portion from the rest of the body.

#### 5. Analysis of variance

This is an important study which shows the variation in animals, in regions and in types of fibres. The interactions between animals and types of fibres, types of fibres and regions and lastly between animals and regions are worked out in Tables VIII and IX dealing with length and diameter respectively. In these calculations the measurements of kemp fibre have been omitted as they are a disqualification. The ewes Nos. 7, 69 and 5 have a different type of coat in different regions and as such they cannot be treated statistically along with the rest.

TABLE VIII  
*Analysis of variance for length†*

Variation due to	Degrees of freedom	Total sum of squares	Mean square	Ratio
Animals	6	11·1208	1·8534	35·63**
Regions	5	7·6900	1·538	29·56**
Types	2	12·1363	6·0681	116·70**
<i>Interactions</i>				
A × T	12	2·7838	0·2319	4·459**
R × T	10	2·3637	0·2363	4·543**
A × R	30	3·8440	0·1281	2·462*
Residual	60	3·1206	0·05201	
Total	125	43·0592		

\* Only when for 5 per cent. \*\* Significant. †Length in inches.

TABLE IX

*Analysis of variance for diameter†*

Variation due to	Degrees of freedom	Total sum of squares	Mean square	Ratio
Animals	6	838.25	139.700	15.79**
Region	5	266.402	53.280	6.017**
Types	2	23147.764	11573.882	1306.0**
<i>Interactions</i>				
A × R	30	187.40	6.246	0.07055
R × T	10	262.284	26.228	2.963**
T × A	12	263.316	21.943	2.478*
Residual	60	351.294	5.854	
Total	125	25316.71		

\* Only when for 5 per cent. \*\* Significant. † Diameter in microns.

Referring to the tables of analysis of variance, it is evident that variation due to animals is significant as predicted already. The regional variation for length measurements is also very significant and the same is true of the types of fibres. This will show clearly the reason why each type of fibre has to be studied independently. The interactions between animals and types, regions and types, and animals and regions are significant. This is expected in the case of length measurements because of the sudden change in the length level of the back wools. This significance in the case of animals and regions is true only at the 5 per cent level. In considering the variation due to interactions between animals and regions for diameter measurements, it will be seen that it is non-significant and that between the types and animals it is significant only at the 5 per cent level. This non-significance is also expected as the types of fibres and their dimensional values are the same for animals and regions. The analysis of variance has shown that the sampling of zones and demarking of the regions is statistically correct.

This will show how essential it is to have a large number of sheep to select so that the desired type can be evolved out of the present heterogeneous mass of the Deccan sheep. However, within the number that is now studied Table X gives the percentages of medullated fibres and wool in individual



sheep shows the degree of variation in the fleece character, and points out the ewes which should be selected for the next stage of improvement.

TABLE X  
*Percentage weight of wool and medullated fibres*

Ewe No.	Wool	Heterotypes	Kemps
4	74.83	14.77	11.26
31	51.78	31.53	6.69
30	80.15	17.11	2.73
41	74.71	20.77	4.52
72	72.30	24.57	3.12
20	84.24	12.75	2.99
89A	67.01	12.95	20.02

Ewe No.	Wool	Medullated fibres
7	42.72 (24.10 fine) (18.62 strong)	57.27
5	51.01 (36.77 fine) (14.24 strong)	48.97
69	57.92 (36.80 fine) (21.12 strong)	42.05

Ewe No. 20 stands first both in regard to medullation and fineness of fleece. Ewe No. 30 stands second although a slightly better in length measurement than the previous one. Ewe No. 72 follows these two in its fleece quality.

#### *6. Method of recording individual and progeny performances*

After studying the wool quality of the individual sheep, it is necessary to record it in such a way that not only will it be easy to find out the life-time performances of that sheep, but the results of its progeny, can be referred to, for further selection or elimination. This subject has been worked out in great detail by Dr Waters, Massey Agriculture College, New Zealand, who was kind enough to supply the farm with the information of his card-index method. Methods of sheep improvement are in an advanced stage in that country and there is yet time to apply the details worked out by Dr Waters to our conditions. However, a suitable method has been framed for recording wool quality of the Deccan sheep (*vide* the form on page 205).



7. *Miscellaneous notes in the study of wool quality*

*Monthly growth of wool in two extreme types of Deccan sheep.*—In the Deccan, shearing is done twice a year. The shepherd believes that the wool breaks off if left over to grow for the whole year. As stated in the previous studies the fleeces of the Deccan sheep are in general of the 'C' grade type segregated on the farm. The difference in wool quality from the point of growth will easily be seen by comparing figs. 1 and 2 on Plate XVII.

It is of great importance to study the problem of breakage of wool in the selection of the stud stock. The continuous growth of wool mainly depends upon the breed and the feed and to some extent on climate. This year a test experiment was carried out on the shoulder wool of the two types of Deccan sheep to study the monthly growth. The ewes were kept without shearing till the end of the year. Samples were taken from the month of July when it was possible to clip the wool properly. It must be noted here that the reading of length for a particular month denotes the growth of the staple of the previous month.

The whole tuft taken as a sample was analysed on the basis of the fibre type as mentioned previously, and every type of fibre was measured for length. A hundred readings for length of each type of fibres were recorded and the mean, standard deviation and coefficients of variation were calculated. These statistical constants are given in Tables XI and XII.

TABLE XI

*Table of constants for monthly growth of length of fibres (non-breaking type ♀ No. 4)*

Months	Fine fibres			Strong fibres			Heterotypes			Kemps		
	Mean	S. D.	C. V.	Mean	S. D.	C. V.	Mean	S. D.	C. V.	Mean	S. D.	C. V.
July	2.41	0.83	34.44	3.17	0.36	11.36	2.51	0.55	21.92	1.68	0.42	25.00
August	2.56	0.84	13.28	3.68	0.35	9.51	2.31	0.61	26.40	1.25	0.31	24.80
September	3.38	0.40	11.84	4.61	0.34	7.38	3.17	0.91	28.70	1.54	0.50	38.32
October	4.06	0.47	11.58	5.22	0.50	9.58	3.33	1.04	31.23	1.96	0.25	12.76
November	4.03	0.41	10.17	5.23	0.61	11.55	2.86	1.31	45.81	1.73	0.44	25.44
December	4.75	0.53	11.16	6.04	0.70	11.59	2.94	1.22	41.51	1.82	0.43	23.62
January	4.55	0.64	14.07	5.79	0.73	12.61	4.09	0.86	21.27	1.78	0.51	28.65

TABLE XII

*Table of constants for monthly growth of length of fibres (breaking type D ♀ No. 44)*

Months	Fine wool fibres			Hairy fibres			Kemps			Remarks
	Mean	S. D.	C. V.	Mean	S. D.	C. V.	Mean	S. D.	C. V.	
July	1.42	0.16	11.19	1.07	0.20	18.69	0.99	0.27	27.28	Month of maximum growth. Month in which shedding takes place.
August	1.62	0.18	11.11	1.03	0.21	20.39	1.14	0.30	26.31	
September	1.31	0.18	9.95	1.22	0.20	16.39	1.13	0.26	22.82	
October	2.20	0.27	12.27	1.05	0.27	25.71	1.10	0.28	26.46	
November	1.56	0.34	21.80	1.06	0.15	14.16	0.89	0.22	25.00	
December	1.40	0.23	20.15	0.85	0.40	47.07	0.97	0.17	17.52	
January	2.07	0.39	18.84	1.12	0.45	40.18	0.94	0.32	34.18	

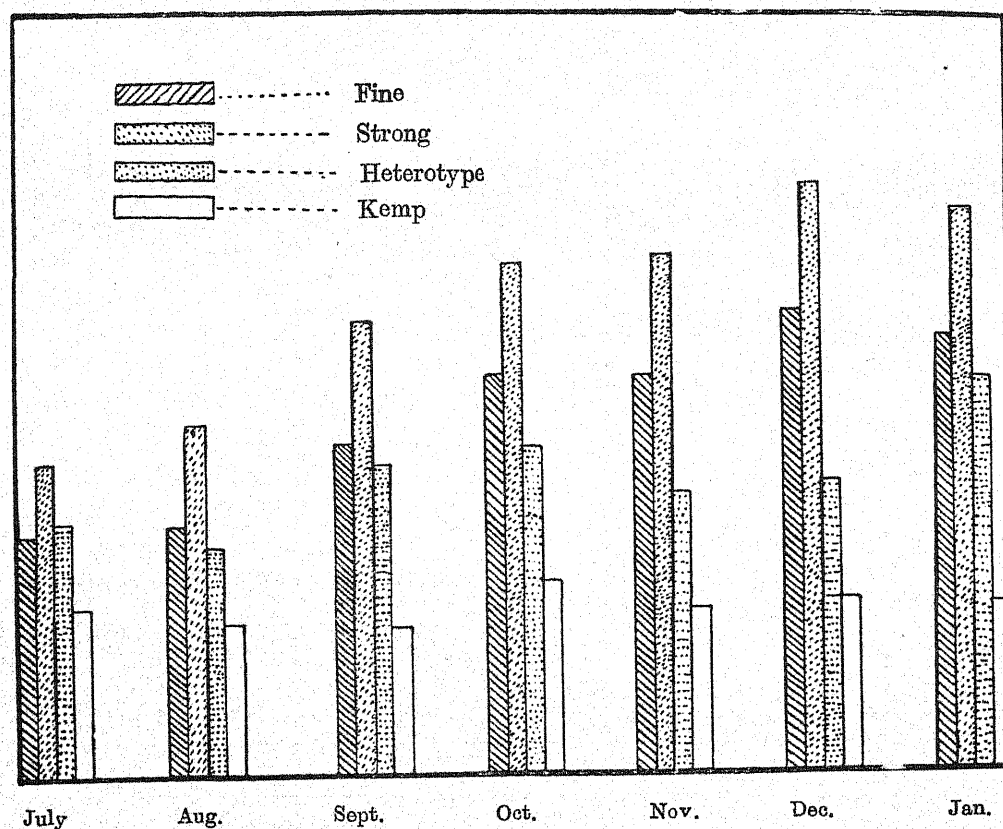


Fig. 6. Monthly growth of fibres of different types in the 'A' grade Deccan sheep.

The above diagrams will clearly show that the 'C' grade wool breaks off in October while in the 'A' grade the growth is slightly retarded. The maximum growth attained in the 'C' grade is 2.22 in. in August, but the staple suddenly breaks in the month of September. It recoups its length only in January, while the fine fibre drops in length from 2.20-1.56-in. in October and then makes a sudden growth to 2.07 in. in December. These results show that the 'C' grade wool breaks off definitely once in a year, while the 'A' grade wool has a distinct tendency of developing into a non-breaking type.

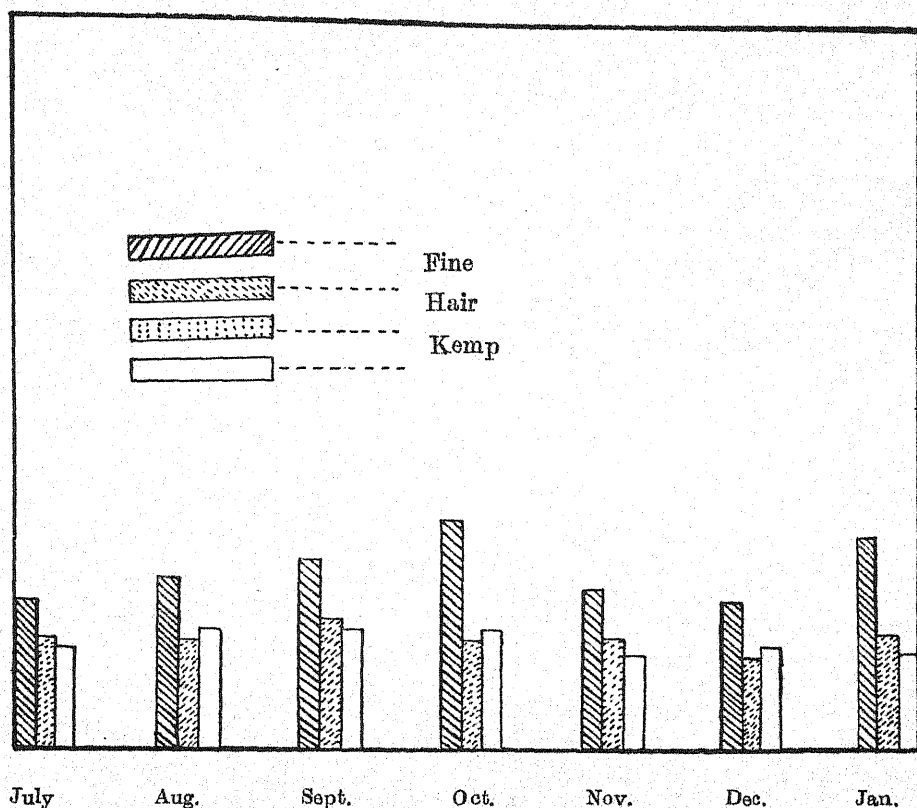


Fig. 7. Monthly growth of fibres of different types in 'C' grade Deccan sheep.

*Percentage of clean wool yield.*—This factor is not of much importance in the case of the Deccan sheep which are washed prior to shearing. But it is necessary to calculate the yield of wool for the Merino and the cross-breds which are highly conditioned. A statement of weights of fleeces from the shearing yard is of little importance in estimating the yield of wool per animal. Dr V. Bosman, Senior Wool Research Officer of South Africa, was kind enough to help the authors with the details on this subject. The method was studied with the help of the apparatus from the Chemistry Research Laboratory of the Agricultural College, Poona. The following yields of wool were estimated for the Merino and cross-bred rams.

Breed	Yield (per cent)
Cross-bred No. 1	49.14
Cross-bred No. 2	65.73
Merino ram	47.63
Merino ewes	49.26

*Percentage of grease.*—The percentage of grease in a fleece is associated with its quality. Secondly, it has a bearing on the feed and environment. It is essential to know the details of grease extent in Merino or cross-bred sheep so that the effect of breeding these in India can be fully gauged. This point of study has been included in the proposed wool-testing branch. The following estimation of grease in the three breeds of sheep on the farm was carried out along with the clean wool yield. These readings are of great interest as a comparison in these breeds on the farm, including the segregated Deccan types.

Breed	Grease content (per cent)
Grade A	4·30 to 4·96
Grade B	3·51 to 4·19
Grade C	2·69 to 2·93
Cross-bred rams	7·11 to 11·48
Merino rams	19·00 to 23·74
Merino ewes	9·40 to 13·29

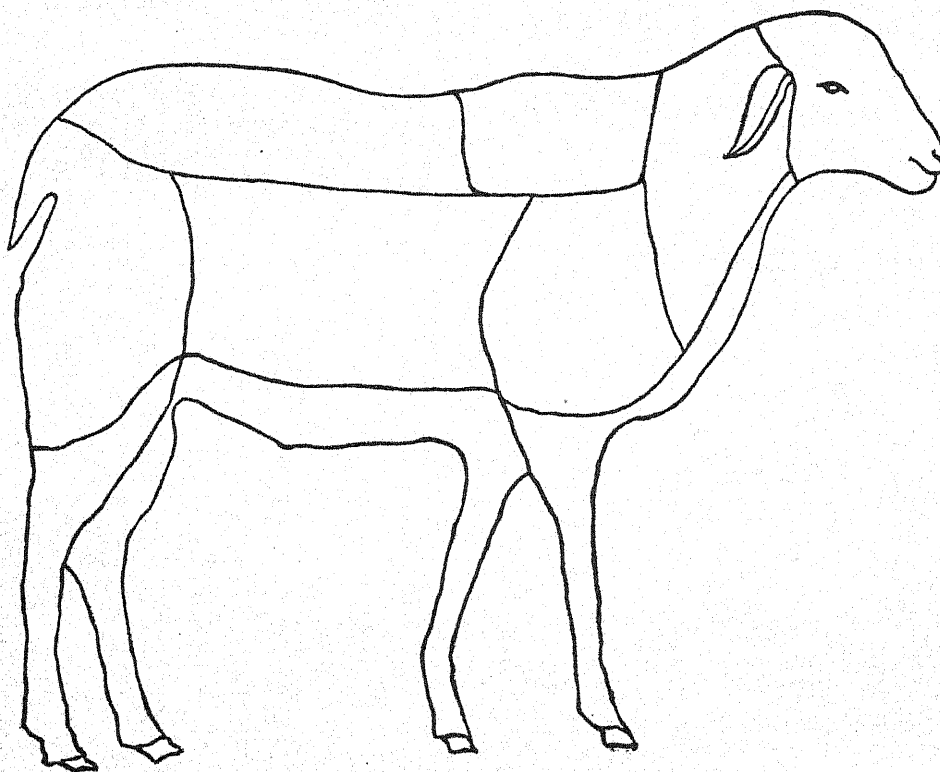
*Density of fleece.*—The density of fleece is expressed as the number of fibres per unit area. This has a direct bearing on the fineness and yield of wool. The details of estimation of density with the help of the 'Wyedena Wool Caliper' was studied from the information and calipers supplied by Dr V. Bosman. The following readings for the three breeds on the farm will be interesting as a comparison.

Breed	Density shoulder wool (per 4 sq. cm.)
Merino ram	20392
Cross-bred ram No. 1	10080
Cross-bred ram No. 2	7109
Deccan ram	2292





## MEDULLATION

Zonal analysis of the  $\frac{\text{Ram}}{\text{Ewe}}$  Regd. No.

# NUTRITIONAL EXPERIMENTS WITH CHICKENS

BY

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## INTRODUCTION

IT is now widely recognized that cereals and their by-products do not alone supply all the necessary nutrients for normal growth in chickens. The feeding of cereals without protein and mineral supplements, especially during the early stages of growth, invariably results in slow and uneven growth, high mortality and poor food utilization. In western countries, where poultry keeping is most highly developed and specialized, it is customary to make good the deficiencies in cereals by supplements of suitable animal or vegetable proteins and minerals.

The poultry industry in India is, however, very different from that in western countries for there is practically no specialization of production, the birds usually being kept in very small flocks under the most primitive conditions of housing, feeding and management. The chickens are hatched under hens and are fed limited amounts of small grains during the first few weeks of their life, but, thereafter, it is not customary to feed any appreciable amounts of food. From a few weeks of age onwards the birds are allowed to roam freely in search of their own food and pick up such materials as household scraps, fallen grains and various forms of small animal life. Such methods of rearing are very unsatisfactory, for the mortality during rearing is usually very high (50 per cent is a common figure) and the rate of growth is very much lower than that obtained under western conditions.

In view of the lack of knowledge regarding chick nutrition under Indian conditions, a series of experiments were carried out to investigate the growth-promoting value of various supplements in the diet of chicks fed on a standard basal diet consisting of locally produced foodstuffs supplemented with green food and crushed limestone.

In view of the proven value of separated milk in the diet of chicks, separated milk was chosen as the control supplement in each experiment and the results obtained were compared with those obtained with the standard diet only and with the standard diet supplemented with soya bean meal plus salt. As the cost of a ration is largely determined by the level of its protein content, an analysis was made of the protein consumptions of all

groups during the different stages of growth. Further, as there was little information available concerning the growth rates of different breeds under Indian conditions, it was decided to use three breeds for the experiments and keep separate records of their growth rates.

### METHODS

*Breeds.*—White Leghorn and Rhode Island Red hatching eggs were purchased from three reputable breeders and *desi* eggs were purchased in the local bazar. Though the *desi* eggs were of unknown origin and age it was found that the hatching results were satisfactory and that the chickens were representative of the *desi* stocks in the neighbourhood.

*Brooding and rearing.*—The chickens were all wing-banded at day-old and transferred from the incubator to a brick-built sectional brooder house, each compartment measuring 10 ft.  $\times$  9 ft. The chicks were brooded under electric brooders measuring  $2\frac{1}{2}$  ft.  $\times$   $2\frac{1}{2}$  ft. The temperature under the brooders was maintained at approximately 90°F. during the early stages of brooding but this was gradually reduced by the manipulation of a thermostatic control fitted on each brooder. The chicks were confined near the source of heat for the first few days by means of a wire guard, but thereafter they were allowed free access to the whole compartment and at one week old they were allowed outside in wire enclosed runs. The chicks were kept under heat for 2-5 weeks according to the prevailing weather conditions but were kept in the brooder house up to 8 weeks in order to prevent losses from crows and other predatory animals. Sand was used as litter throughout the brooding stage.

At 8 weeks of age the chickens were transferred to 6 ft.  $\times$  3 ft. rearing houses constructed from  $\frac{1}{2}$  in. angle iron covered with wire ( $\frac{1}{2}$  in. mesh) on all four sides and roofed with an asbestos sheet. Further protection from the rain and sun was provided by means of *chuppars* made of bamboo and *bhurra* grass. Each rearing house was placed centrally in a run measuring 40 ft.  $\times$  40 ft. surrounded by 6 ft. wire netting.

### FEEDING

All the birds were fed on mash and grain rations from day-old onward. The mash was fed *ad libitum* in suitable containers and the grain was fed in the litter, morning and evening, according to appetite. Supplementary calcium, as broken limestone, was fed *ad libitum* from day-old onward. The birds also received succulent green food, such as alfalfa or berseem, twice daily.

A basal mash consisting of wheat bran 50 parts, yellow maize meal 30 parts and finely ground oats 20 parts was fed throughout to all the experimental groups. During the first 8 weeks the grain ration consisted of equal parts of yellow broken maize, *jowar* and millet (*cheena*) and from 8 weeks onwards the grain mixture consisted of equal parts of yellow cracked maize, wheat and paddy. Table I gives the average composition of the mashes and grains used in the experiments.

TABLE I

*Food composition (percentages) on dry matter basis*

Feeding stuff		Fibre	Crude protein	Ether extract	Nitrogen-free extract	Ash
<i>Basal mash—</i>						
	Parts					
Wheat bran	50	} 12·5	11·4	5·9	63·5	6·7
Yellow maize	30					
Ground oats	20					
<i>Soya bean mash—</i>						
	Parts					
Basal mash	81·5	} 11·3	15·9	7·7	58·2	6·9
Soya bean	18					
Salt	0·5					
<i>Chicks' grain—</i>						
	Parts					
Yellow maize	1	} 5·8	9·9	4·2	77·4	2·7
Cheena	1					
Jowar	1					
<i>Grower's grain—</i>						
	Parts					
Yellow maize	1	} 4·6	9·7	3·1	79·5	3·1
Paddy	1					
Wheat	1					



*Experiment 1. To investigate the value of separated milk supplements to cereal rations*

*Stock.*—A total of 84 day-old chicks, hatched in January, 1939, were wing-banded and weighed individually at hatching and divided into two comparable groups as follows :—

Breed	Group 1	Group 2
	No. of chicks	No. of chicks
White Leghorn	28	28
Rhode Island Red	14	14
Total	42	42

*Feeding.*—Both the groups were fed on the standard basal rations described above. Group 1 received the basal ration *plus* separated milk only to drink from day-old to 10 weeks. From 10-24 weeks separated milk and water in separate containers were fed. Group 2 was fed water only to drink from 0-6 weeks, separated milk only from 6-8 weeks, water only from 8-12 weeks and separated milk and water from 12-24 weeks. Originally it was proposed to keep Group 2 on water only to drink throughout the whole experimental period but the condition of the chicks necessitated the alterations described above.

*Rate of growth.*—The chickens were weighed individually at each weighing and the average weights of the cockerels and pullets in each group are given in Table II. For purposes of convenience Group 1, fed on separated milk from day-old, is hereafter known as 'the milk-fed group' and Group 2 as 'the water-fed group'. The cockerels were removed from the experiment at 14 weeks.

TABLE II

*Average weights in ounces for each breed*

Breed	White Leghorn				Rhode Island Red			
	1		2		1		2	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Day old	1.48	1.46	1.47	1.47	1.42	1.44	1.48	1.41
1 week	2.4	2.2	1.7	1.8	2.3	2.2	1.8	1.7
2 weeks	4.2	3.9	2.2	2.2	4.2	4.1	2.1	2.1



Breed	White Leghorn				Rhode Island Red			
Group	1		2		1		2	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
3 weeks	6.1	5.7	2.5	2.6	6.1	5.8	2.6	2.7
4 "	9.4	8.5	3.0	3.0	9.1	8.9	2.7	3.0
5 "	13.3	12.6	3.1	3.3	13.4	13.1	2.7	3.4
6 "	15.8	14.8	3.1	3.8	16.6	16.3	3.0	3.9
7 "	21.5	20.5	5.6	5.9	22.4	21.1	5.0	6.4
8 "	26.1	24.6	8.0	8.5	27.9	25.2	7.2	9.1
9 "	30.7	28.8	9.5	9.6	33.5	28.2	8.6	10.3
10 "	37.0	34.6	10.2	10.2	41.3	34.5	9.4	11.2
12 "	46.0	40.8	13.1	13.9	49.5	41.2	14.5	16.6
14 "	52.0	47.5	21.7	22.0	59.2	49.2	23.3	25.0
16 "		52.4		30.4		56.2		32.0
18 "		55.7		35.6		60.2		38.7
20 "		57.1		36.8		57.5		41.3
22 "		61.0		41.2		60.5		43.3
24 "		63.6		46.0		65.5		48.0

Table II shows very clearly the marked difference in the rate of growth obtained from the birds on the two rations under experiment. The birds on the milk ration made satisfactory gains throughout, whilst the birds on the water ration were considerably lower in weight at each weighing from one week onward. At 6 weeks the average weights of the White Leghorn cockerels and pullets on the milk diet were 15.8 oz. and 14.8 oz. respectively, whereas the corresponding figures for the birds on the water diet were 3.1 oz. and 3.8 oz. The corresponding figures for the Rhode Island Reds on the milk and water rations were 16.6 oz., 16.3 oz., 3.0 oz., and 3.9 oz. respectively. The gains made by the birds of both breeds on the milk ration compare very favourably with data published by Card and Kirkpatrick [1918], Charles and Knandal [1928] and Kempster and Parker [1936] but the rate of growth of the water-fed group is decidedly sub-normal, especially during the period 4-6 weeks. At 6 weeks the birds in the water-fed group were all very weak and, as it was apparent that they would not live much longer on the ration, it was decided to supplement their diet with separated milk. From 6-8 weeks the condition of the birds in Group 2 improved and the gains in weight were significantly higher than during the previous periods. From 8-12 weeks water was again fed in place of milk to Group 2 and, as again there was a falling off in results, it was decided to feed milk and water to this group for the remainder of the experiment. The rate of growth in Group 2, when fed on milk from 12 weeks onward, improved very considerably and the average gain made from 12-24 weeks was even greater than the corresponding figure in Group 1, which was fed on milk from day-old. The average final weights of the White Leghorn pullets at 24 weeks were 63.6 oz. in the milk-fed group and

46.0 oz. in the water-fed group. The corresponding figures for the Rhode Island Reds were 65.5 oz. and 48.0 oz. respectively.

*Food consumption.*—Table III gives the average food consumption per bird in pounds for each group for each period of 4 weeks and the food consumption in pounds per pound of live weight gain.

TABLE III  
*Food consumption*

	Average food consumption per bird (lb.)		Food consumption in lb. per pound live weight gain	
	Group 1	Group 2	Group 1	Group 2
1. 0-4 weeks	1.71	1.15	3.70	22.20
2. 4-8 "	4.44	1.40	3.74	6.32
3. 8-12 "	5.53	3.24	5.05	12.38
4. 12-16 "	4.77	4.01	8.22	5.32
5. 16-20 "	4.61	4.21	26.50	6.99
6. 20-24 "	5.32	4.90	8.75	9.50

NOTE.—The food consumption figures include the separated milk reckoned on a dry matter basis of 9 per cent.

The average food consumption per bird was greater during each periods for the milk-fed group than the water-fed group. During the first three periods the milk-fed group made much better utilization of their food, as shown by the food consumed per unit of live weight gain. During period 1 the food consumptions per unit of gain were 3.7 and 22.2 for Groups 1 and 2 respectively. During period 2 the corresponding figures for Groups 1 and 2 were 3.74 and 6.32. The improved food utilization by Group 2 during period 2 was solely due to the addition of the milk during the second half of the period. In period 3 Group 1 consumed 5.05 lb. of food per lb. of live weight gain whereas the figure for Group 2, which was again on water during the whole period, was 12.3. During periods 4 and 5 Group 2 made better utilization of the food consumed than Group 1 but during these periods both groups were fed similar diets. The high food consumptions per unit of gain for Group 1 during these periods is quite normal for birds which have received adequate rations during the early stages of growth. At 12 weeks of age the birds of Group 1 were well grown for their age and the efficiency of food utilization as regards growth normally decreases very materially during the latter stages of growth. The birds in Group 2 at 12 weeks of age were, however,

very much sub-normal in weight and it is quite normal for animals to make better utilization of their food when fed on a good diet after being fed on a deficient one. The exceptionally high food consumption per unit of live weight gain made by Group 1 in period 5 is most probably due to the birds being well grown for their age at 16 weeks and a subsequent check in the rate of growth from 16-20 weeks due to a spell of hot weather. On the other hand the birds in Group 2 were undersized at 16 weeks and made relatively better gains in weight despite the rise in temperature.

*Milk consumption.*—Table IV gives details of the milk and water consumed by both groups during the different stages of the experiment. In Group 1 the milk and water consumption was measured throughout all stages but in Group 2 the milk and water was only measured during the periods that the birds were given supplements of milk.

TABLE IV

*Average consumption (in lb.) per chick of milk and water*

Period	Group 1		Group 2	
	Average milk consumption per chick	Average water consumption per chick	Average milk consumption per chick	Average water consumption per chick
1. 0-4 weeks	2.48	<i>Nil</i>	<i>Nil</i>	Unrecorded
2A. 4-6    ,,	2.21	<i>Nil</i>	<i>Nil</i>	Do.
2B. 6-8    ,,	2.48	<i>Nil</i>	0.97	<i>Nil</i>
3A. 8-10   ,,	3.29	<i>Nil</i>	<i>Nil</i>	Unrecorded
3B. 10-12   ,,	1.01	3.70	<i>Nil</i>	Do.
4. 12-16   ,,	2.31	9.42	3.82	4.51
5. 16-20   ,,	6.06	17.20	6.71	15.00
6. 20-24   ,,	7.84	14.49	6.56	17.97

The average milk consumption per chick in Group 1 from 0-10 weeks whilst receiving milk only to drink, was 10.46 lb. From 10-12 weeks, whilst fed on milk and water, the average consumptions of milk and water were 1.01 lb. and 3.70 lb. respectively. During period 4 the proportion of milk to water consumed was even less but during periods 5 and 6 the proportions of milk to water consumed were greater than in periods 3B and 4. The

total milk consumption from 0-24 weeks was 27.68 lb. Group 2, during period 4, consumed 3.82 lb. of milk and 4.51 lb. of water. The proportions of milk to water consumed during periods 5 and 6 decreased.

*Protein consumption.*—Table V gives details of the total protein percentages contained in the food consumed during the different periods of the experiment. The milk was brought to a dry matter basis and included along with the mash and grain figures.

TABLE V

*Protein consumption*

Period	Group 1		Group 2	
	Supplement	Percentage of protein	Supplement	Percentage of protein
1. 0-4 weeks	Milk only	15.6	Water	11.1
2A. 4-6 "	Do.	13.2	Do.	11.0
2B. 6-8 "	Do.	13.3	Milk only	13.3
3A. 8-10 "	Do.	13.4	Water	10.8
3B. 10-12 "	Milk & water	11.6	Do.	10.8
4. 12-16 "	Do.	11.9	Milk & water	13.0
5. 16-20 "	Do.	13.3	Do.	14.3
6. 20-24 "	Do.	14.3	Do.	13.6

In Group 1, the percentage of protein in the food consumed was at its maximum (15.6) during period 1. From 4-10 weeks the percentage of protein consumed was comparatively steady, ranging from 13.2 for period 2A to 13.4 for period 3A. The percentage of protein consumed during period 3B was only 11.6 but thereafter the consumption rose each period to give a figure of 14.3 for period 6. The percentages of protein in the food consumed in Group 2 were 11.1 and 11.0 for periods 1 and 2A. The protein consumption rose to 13.3 per cent during period 2B, whilst the birds received milk only to drink, but fell to 10.8 per cent during periods 3A and 3B when given water only to drink. During period 4 the percentage of protein consumed rose to 13.0 but after rising to 14.3 in period 5 there was a fall to 13.6 in period 6.



*Mortality.*—The value of separated milk in the diet of the chick is very well illustrated in Table VI which gives a summary of the number of deaths which occurred in each group at different stages of the experiment.

TABLE VI

*Mortality (0-24 weeks)*

Period	Number of deaths	
	Group 1	Group 2
1. 0-1 week	1	2
2. 1-2 weeks	..	3
3. 2-3 ..	1	2
4. 3-4 ..	..	4
5. 4-5 ..	..	4
6. 5-6 ..	..	5
7. 6-7 ..	..	1
8. 7-8 ..	..	1
9. 8-11 ..	..	..
10. 11-12 ..	..	2
11. 12-13 ..	..	1
12. 13-14 ..	..	1
13. 14-24 ..	..	..
Total mortality	2	26
Percentage mortality	4.8	61.9

Group 1, fed on milk, had an excellent health record throughout. The birds always handled well, had good tight feathers and were always ready for their grain. The total mortality of 2, representing 4.8 per cent of the chicks used in the experiment, may be considered very satisfactory. Group 2, however, had a very poor health record; the chicks were excessively thin until very late in the experiment, had ruffled feathers and abnormal appetite. From a few days onward the chicks showed little interest in their food and spent most of the day scratching feverishly in their runs. The birds



showed the typical symptoms of depraved appetite, consuming such objects as nails, pieces of wire and paper. The mortality from 0-6 weeks, whilst on water, was 20 chicks or 47·6 per cent, which is very much in excess of the normal. The birds pick'd up very considerably from 6-8 weeks, when fed on milk in place of water, and only two deaths occurred during this period. From 8-12 weeks, the birds again fell back in condition and two more deaths occurred during the 11-12th week. From 12-24 weeks, when fed on milk and water, the condition of the birds improved very rapidly and no deaths occurred after the 14th week. The total percentage mortality from 0-24 weeks of 61·9 was very much in excess of the normal. All the birds which died were very much emaciated and the intestines were usually almost void of food. The gizzard contents were abnormal in that they contained excessive quantities of such materials as wire, nails and glass, all indicative of depraved appetite. The gizzard linings were very much eroded and darkened in colour suggesting that the diet of the birds was deficient in the gizzard factor, which is essential for normal growth of the gizzard.

*Egg production.*—Table VII gives details of the age at first egg, the percentage of birds laying at 24 weeks and the average egg production and egg size per bird for each breed on the two rations under test.

TABLE VII  
*Egg production*

Group	1		2	
Breed	White Leghorn	Rhode Island Red	White Leghorn	Rhode Island Red
Age at first egg (days)	137	134	159	215
Percentage of birds laying at 24 weeks	42·8	16·6	20·0	0·0
Average number of eggs per bird to 24 weeks	10·1	12·9	0·83	0·0
Average egg size (oz.)	1·61	1·51	1·55	0·0

The first eggs in the milk-fed group were produced at 134 and 137 days by the Rhode Island Reds and White Leghorns respectively. The corresponding figures in Group 2 were 215 and 159 days. In the milk-fed group, 42·8 per cent of the White Leghorns and 16·6 per cent of the Rhode Island Reds had started to lay at 24 weeks, whereas in Group 2 only 21 per cent of the Leghorns and none of the Rhode Island Reds had started to lay. At 24 weeks the average egg production per bird in Group 1 was 10·1 for the White Leghorns and 12·9 for the Rhode Island Reds. The corresponding figures for the water-fed group were 0·83 and nil for the Leghorns and Rhode Island Reds respectively.

*Conclusions.*—(1) The feeding of a ration consisting of cereals plus liberal amounts of calcium and green food to chickens resulted in very poor growth, high mortality and very poor food utilization.

(2) The cereal ration appeared to be deficient in a factor necessary for the normal growth of the gizzard.

(3) Cereals supplemented by calcium, green food and separated milk, instead of water, to drink gave excellent growth results from 0-10 weeks.

(4) From 10 weeks onwards the basal ration plus separated milk and water to drink gave satisfactory growth results.

(5) Birds receiving milk to drink came into lay considerably earlier than birds on the basal ration only.

### *Experiment 2*

The results obtained in Experiment 1 demonstrated the value of supplementing cereal rations with separated milk but gave no information concerning the optimum amount of milk that should be fed to give maximum results. As milk is a somewhat expensive article of diet, a second experiment was conducted to throw more light on this important problem and to see if the chicks would balance up their ration effectively when separated milk and water were fed in separate containers.

*Stock.*—A total of 172 day-old chicks hatched in January, 1939 were wing-banded and divided up into two similar groups as follows :—

Breed	Number of chicks	
	Group 3	Group 4
<i>Desi</i>	27	27
White Leghorn	29	28
Rhode Island Red	31	30
Total	87	85

*Feeding.*—Both the groups were fed on the basal rations used in Experiment 1.

Group 3 was fed the basal ration and separated milk only to drink during the first 8 weeks and separated milk and water in separate containers from 8-24 weeks. Group 4 was fed separated milk and water in separate containers from day-old onward. The methods of feeding and management adopted were similar to those used in Experiment 1 but in this instance the cockerels were removed from the experiment at 12 weeks.

*Rate of growth.*—Table VIII gives a statement of the average weights in ounces of the cockerels and pullets of each breed at weekly intervals from 0-8 weeks and at bi-weekly intervals from 8-24 weeks.

TABLE VIII

*Average weights in ounces for each breed*

Breed	White Leghorn				Rhode Island Red				Dasi			
	3		4		3		4		3		4	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day old	1.52	1.46	1.50	1.49	1.48	1.46	1.47	1.49	1.11	1.11	1.05	1.13
1 week	2.34	2.03	2.08	1.85	2.23	1.96	1.84	1.86	1.67	1.67	1.50	1.55
2 weeks	4.3	3.8	3.6	3.0	4.5	3.8	3.1	3.0	3.1	3.2	2.6	2.5
3 "	6.0	5.2	5.3	4.5	5.5	5.2	4.9	4.3	4.0	4.3	3.6	3.7
4 "	9.1	7.8	7.4	6.9	8.6	7.6	6.6	5.5	6.1	6.3	5.3	5.2
5 "	12.1	10.3	10.4	9.8	11.0	10.3	8.7	7.7	8.5	8.6	7.9	7.4
6 "	16.6	13.8	12.9	12.1	14.6	13.6	12.5	10.4	11.6	11.7	10.0	9.8
7 "	21.6	17.6	17.6	15.8	18.5	17.7	15.9	13.1	15.1	15.1	13.3	13.1
8 "	23.0	22.4	21.7	19.2	24.2	23.0	19.5	16.1	19.6	19.4	15.0	15.7
10 "	35.1	29.3	31.5	28.2	32.4	29.2	29.9	23.6	28.9	25.4	23.8	22.6
12 "	41.6	38.0	42.9	35.0	40.0	35.8	40.8	32.0	33.2	31.6	32.2	30.9
14 "		41.0		40.2		39.6		36.5		36.1		34.1
16 "		41.6		40.6		40.8		38.7		37.5		36.7
18 "		45.5		43.4		45.1		43.2		41.2		39.7
20 "		50.7		51.4		51.1		50.8		46.7		45.6
22 "		57.8		55.4		58.2		58.2		49.4		48.3
24 "		62.0		62.0		61.3		62.5		50.8		50.8

The weights of the cockerels and pullets of all the three breeds were greater in Group 3 than in Group 4 at each weekly weighing from 1-8 weeks of age. The differences in weights between the corresponding birds in the two groups became consistently greater at each weekly weighing so that at 8 weeks the birds in Group 3 were significantly heavier than those in Group 4. At 8 weeks the average weights of the White Leghorn, Rhode Island Red and *desi* males were 28.0, 24.2 and 19.6 oz. respectively, whilst the corresponding figures in Group 4 were 21.7, 19.5 and 15.0 oz. The average weights of the females in Group 3 at 8 weeks were White Leghorns 22.4 oz., Rhode Island Reds 23.0 oz. and *desis* 19.4 oz., whilst in Group 4 the corresponding weights were 19.2, 16.1 and 15.7 oz. respectively. From 8 weeks onwards, whilst both groups were fed on the same rations, Group 4 grew slightly faster than Group 3. At 12 weeks of age, when the birds were sexed, there were no significant differences between the weights of the cockerels and pullets of Group 3 and the corresponding birds in Group 4 except in the case of the Rhode Island Red pullets, which were still significantly heavier in Group 3. From 12-24 weeks of age the rate of growth of the pullets of each breed was very similar in both groups for each period of the experiment. At 24 weeks the average weights of the pullets in Group 3 were White Leghorns 62.0 oz., Rhode Island Reds 61.3 oz. and *desis* 50.8 oz. The corresponding figures in Group 4 were 62.0, 62.5 and 50.8 oz.

*Food consumption.*—Table IX gives the average food consumption per bird in pounds for each group for each period of 4 weeks and the food consumption in pounds per pound of live weight gain.

TABLE IX

*Food consumption*

Period	Average food consumption per bird (lb.)		Food consumption in lb. per pound live weight gain	
	Group 3	Group 4	Group 3	Group 4
1. 0-4 weeks	1.46	1.54	3.88	5.77
2. 4-8 "	3.07	2.92	3.05	3.63
3. 8-12 "	5.03	5.05	6.52	4.86
4. 12-16 "	4.28	4.68	8.54	9.45
5. 16-20 "	3.85	4.35	7.00	7.78
6. 20-24 "	5.14	4.74	8.85	10.20

The average food consumption was fairly similar for each period for both groups. During periods 1 and 2 Group 3 made better utilization of the food consumed than Group 4 but in period 3 Group 4 made better utilization than



Group 3. The better utilization of the food consumed during periods 1 and 2 by Group 3 is due to the fact that the birds consumed practically the same amounts of food as Group 4 but made better gains in weight during each period. During period 3, the birds in Group 4 made greater gains in weight than Group 3 without a corresponding increase in food consumption. From 12 weeks onwards the food consumptions were somewhat erratic and no conclusion can be drawn from the results obtained. The average food consumptions per bird in Groups 3 and 4 from 0-24 weeks were 22.83 lb. and 23.28 lb. respectively.

*Milk consumption.*—Table X gives details of the milk and water consumptions by both groups during the different stages of the experiment but in period 6 no record was kept of the water consumed as it was impossible to measure it accurately owing to the onset of the monsoon.

TABLE X

*Average consumption (in lb.) of milk and water*

Period	Group 3		Group 4	
	Average milk consumption per chick (lb.)	Average water consumption per chick (lb.)	Average milk consumption per chick (lb.)	Average water consumption per chick (lb.)
1. 0-4 weeks	1.68	<i>Nil</i>	0.99	0.63
2. 4-8 "	4.74	<i>Nil</i>	2.84	1.78
3. 8-12 "	2.18	5.66	3.81	1.93
4. 12-16 "	4.28	11.08	5.17	9.57
5. 16-20 "	6.25	15.05	7.19	13.33
6. 20-24 "	7.34	Unrecorded	7.90	Unrecorded

The average milk consumption in Group 3 from 0-8 weeks, when receiving milk only, was 6.42 lb. During period 3, whilst fed on both milk and water, the average consumptions of milk and water per chick were 2.18 lb. and 5.66 lb. respectively. During periods 4 and 5 there was a very considerable rise in the milk consumption but there was also a corresponding rise in the water consumption. During period 6 the milk consumption per chick rose to 7.34 lb. but unfortunately it was impossible to measure the water consumption as the birds consumed water from pools in the runs.

During periods 1 and 2 Group 4 consumed appreciably less milk than Group 3 but this was offset by the birds consuming considerable quantities



of water. The total consumptions of milk and water from 0-8 weeks were 3.83 lb. and 2.41 lb. respectively. During period 3, the milk consumption was greater than that of Group 3 but the birds consumed proportionately less water. During the subsequent periods the proportions of water to milk consumed gradually decreased but in each period the consumption of milk was relatively higher than that for Group 3. The total average consumption of milk from 0-24 weeks in Group 4 was 27.9 lb., whilst in the same period the average consumption by Group 3 was 26.47 lb.

*Protein consumption.*—Table XI gives details of the total protein percentages contained in the food consumed for the different periods from 0-24 weeks.

TABLE XI

*Protein consumption*

Period	Group 3	Group 4
	Percentage of protein	Percentage of protein
1. 0-4 weeks	13.8	12.8
2. 4-8 ..	14.8	13.2
3. 8-12 ..	12.0	12.9
4. 12-16 ..	13.4	13.7
5. 16-20 ..	14.8	15.7
6. 20-24 ..	14.7	14.7

The protein consumption in Group 3 rose from 13.8 per cent in period 1 to 14.8 per cent in period 2. In period 3, when fed on water as well as milk, the protein consumption fell to 12.0 per cent but rose to 13.4 per cent in period 4 and 14.8 per cent in period 5. The protein consumption in period 6 of 14.7 per cent was practically the same as that in period 5. In Group 4, the protein consumption remained fairly steady during the first three periods, the figures being 12.8 per cent, 13.2 per cent and 12.9 per cent. The protein consumptions in periods 4 and 5 were 13.7 per cent and 15.7 per cent respectively but declined to 14.7 per cent in period 6.

*Mortality.*—Table XII gives a statement of the mortality which occurred in Groups 3 and 4 during different stages of the experiment.

TABLE XII

*Mortality*

Period	Deaths	
	Group 3	Group 4
1. 0-1 week	3	4
2. 1-2 weeks	1	3
3. 2-3 "	..	5
4. 3-4 "	3	4
5. 4-6 "	..	1
6. 6-7 "	..	3
7. 7-24 "	1	2
Total mortality	8	22
Percentage mortality	9.3	25.9

Group 3 maintained good health throughout and the total mortality of 8 chickens, representing 9.3 per cent, was quite satisfactory. Seven of the eight deaths occurred during the first four weeks. Group 4 had not as good a health record and the total mortality of 22 chickens, representing 25.9 per cent, is somewhat higher than normal. Though the major part of the mortality also occurred during the first four weeks, deaths at the later stages were more frequent than in the case of Group 3. During the first eight weeks of the experiment the chickens in Group 3 handled better, were better feathered and more alert than those in Group 4 but in the later stages there were no appreciable differences in appearance between the two groups.

*Egg production.*—Table XIII gives particulars in regard to the age at first egg, the percentage of birds laying at 24 weeks and the average egg production and size for each breed in both groups.

TABLE XIII

*Egg production*

Group	3			4		
	White Leg-horn	Rhode Island Red	Desi	White Leg-horn	Rhode Island Red	Desi
Age at first egg (days)	154	174	137	184	187	136
Percentage of birds laying at 24 weeks	27.3	0	72.7	0	0	53.8
Average number of eggs per bird to 24 weeks	1.2	0	12.3	0	0	7.4
Average egg weight (oz.)	1.47	0	1.28	0	0	1.26

In Group 3 the first eggs were produced by the White Leghorns, Rhode Island Reds and *desis* at 154, 174 and 137 days respectively. In Group 4, the corresponding figures were 184, 187 and 136 days. In Group 3, 27.3 per cent of the Leghorns and 72.7 per cent of the *desis* had started to lay by 24 weeks, whereas none of the Leghorns and 53.8 per cent of the *desis* had produced eggs in Group 4. At 24 weeks the average egg production per bird in Group 3 was 1.2 for the Leghorns and 12.3 for the *desis*. The corresponding figures for Group 4 were nil for the Leghorns and 7.4 for the *desis*.

*Conclusions.*—(1) From 0-8 weeks the feeding of a basal ration consisting of cereals plus liberal amounts of green food and calcium supplemented by separated milk only to drink gave better results than the same basal ration plus free choice of milk and water to drink.

(2) From 8 weeks onwards birds given free choice of milk and water to drink made satisfactory gains in weight even though consuming more water than milk.

(3) Birds fed on milk only to drink from 0-8 weeks and milk and water to drink from 8 weeks onwards came into production earlier than birds fed on milk and water from day-old onward.

(4) The feeding of milk and water from day-old onward instead of milk only from 0-8 weeks and milk and water from 8 weeks onwards did not result in a saving in milk over a period of 24 weeks.

### Experiment 3

Though Experiments 1 and 2 demonstrated that the cereals and separated milk gave excellent growth results, low mortality and economic food utilization, further nutritional experiments were considered necessary with other protein supplements, as milk is not always available at economic rates. Experiment 3 was designed to test out the value of a vegetable protein and salt against separated milk in the ration of chicks from day-old to 24 weeks. Soya bean meal was selected in preference to other protein supplements, as it is generally considered to be one of the best of the vegetable proteins.

*Stock.*—A total of 254 day-old chicks hatched in February, 1939, were wing-banded and divided up into 2 comparable groups as follows:—

Breed	Number of chicks	
	Group 5	Group 6
White Leghorn	40	40
Rhode Island Red	34	33
<i>Desi</i>	54	53
Total	128	126

*Feeding.*—Both the groups were fed on the basal rations used in the previous experiments. Group 5 was fed the basal ration plus separated milk to drink from day-old to 6 weeks and separated milk and water to drink in separate containers from 6-24 weeks. Group 6 was given water to drink from day-old onward but the basal mash was supplemented by 18 per cent soya bean meal and 0.5 per cent common salt. The mash fed to Group 6, therefore, contained 81.5 per cent of the basal mash, 18 per cent soya bean meal and 0.5 per cent salt.

*Rate of growth.*—Table XIV gives a statement of the average weights in ounces of the males and females of each breed for each group at different stages of the experiment. The cockerels were removed from the experiment at 10 weeks.

From 0-6 weeks both sexes of all the three breeds fed on the separated milk ration made better weekly gains in weight than the corresponding birds on the soya bean meal ration. At 6 weeks of age the average weights of the White Leghorn, Rhode Island Red and *desi* males were 16.4, 19.9 and 11.8 oz. respectively, whilst the corresponding figures for the birds on the soya bean ration were 11.1, 14.3 and 9.8 oz. The average weights of the females in Group 5 were White Leghorns 15.2 oz., Rhode Island Reds 16.7 oz. and *desis* 11.4 oz., whilst in Group 6 the corresponding figures were 9.5, 11.6 and 7.5 oz.

From 6 weeks onwards the birds of Group 5 fed on separated milk and water made better gains in weight during practically every period than the corresponding birds on the soya bean ration. At 24 weeks the average weights of the pullets in Group 5 were White Leghorns 56.1 oz., Rhode Island Reds 69.5 oz. and *desis* 46.1 oz. The corresponding figures for Group 6 were 46.6, 55.3 and 37.2 oz.

*Food consumption.*—Table XV gives the average food consumption per bird for each group for each period of the experiment and the food consumption in pounds per pound of live weight gain.

The food consumptions were greater in Group 5 for each period than in the corresponding periods for Group 6. However, these figures of food consumption during the periods are not directly comparable in that the birds in Group 5 were considerably heavier than the birds in Group 6 at all periods and thus required more food both for maintenance and growth.

In Group 5, period 1, the food consumption of 3.57 lb. per lb. of live weight gain was considerably lower than the corresponding figure of 4.96 lb. for Group 6. The food utilization figures for the other periods were fairly comparable for both groups.

*Protein consumption.*—Table XVI gives details of the total protein percentages in the food consumed by both groups during the different periods.

Group 5, whilst receiving milk only to drink, consumed 14.1 per cent protein during period 1 and 15.9 per cent protein during period 2B. From 6 weeks onwards, when receiving milk and water to drink, the protein consumption fell to 13.1 per cent in period 2B and 12.8 per cent in period 3. The protein consumptions during periods 4, 5 and 6 were 13.2 per cent, 14.0 per cent and 13.8 per cent respectively.



TABLE XIV  
Average weights in ounces for each breed

Breed	White Leghorn								Rhode Island Red				Deli			
	5				6				5				6			
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		
Day old	1.49	1.47	1.48	1.50	1.61	1.58	1.62	1.60	1.62	1.60	1.03	1.04	1.02	1.05		
1 week	2.8	2.8	2.1	2.0	3.3	2.7	2.4	2.2	2.4	2.2	2.0	2.0	1.6	1.5		
2 weeks	4.4	4.2	3.3	3.0	4.7	4.2	3.6	3.2	3.6	3.2	2.8	2.9	2.4	2.2		
3 "	6.2	6.3	5.1	4.6	7.3	6.4	5.9	5.0	5.9	5.0	4.2	4.3	3.7	3.3		
4 "	9.1	8.9	6.8	6.0	10.7	9.5	8.2	6.8	8.2	6.8	6.2	6.2	5.1	4.6		
5 "	12.5	12.0	8.6	7.5	14.8	12.6	10.8	9.0	10.8	9.0	8.7	8.7	6.7	5.8		
6 "	16.4	15.2	11.1	9.5	19.9	16.7	14.3	11.6	14.3	11.6	11.8	11.4	9.8	7.5		
8 "	23.3	20.2	16.2	13.6	27.7	22.8	21.5	17.2	21.5	17.2	15.8	15.5	13.6	11.2		
10 "	31.4	27.0	24.0	19.5	36.2	29.3	30.5	23.8	30.5	23.8	23.6	20.9	19.4	16.0		
12 "		29.0		21.9		32.7		26.9		26.9		23.8		17.8		
14 "		32.7		24.6		36.2		30.7		30.7		27.9		20.8		
16 "		38.9		29.7		43.5		36.5		36.5		33.3		25.0		
18 "		44.7		35.4		51.2		43.8		43.8		38.0		29.4		
20 "		48.8		38.7		56.2		47.6		47.6		40.6		32.0		
22 "		54.1		43.1		63.9		51.8		51.8		44.9		36.0		
24 "		56.1		46.6		69.5		55.3		55.3		46.1		37.2		



TABLE XV

*Food consumption*

Period	Average food consumption per bird (lb.)		Food consumption in lb. per pound live weight gain	
	Group 5	Group 6	Group 5	Group 6
1. 0-4 weeks	1.46	1.35	3.57	4.96
2. 4-8 „	3.09	2.33	4.10	4.43
3. 8-12 „	3.61	2.93	5.18	4.89
4. 12-16 „	3.53	2.88	5.41	6.01
5. 16-20 „	4.34	3.83	7.03	7.02
6. 20-24 „	5.15	4.37	10.74	11.10

TABLE XVI

*Protein consumption*

Period	Percentage of protein	
	Group 5	Group 6
1. 0-4 weeks	14.1	14.5
2A. 4-6 „	15.9	14.4
2B. 6-8 „	13.1	14.8
3. 8-12 „	12.8	14.4
4. 12-16 „	13.2	13.9
5. 16-20 „	14.0	13.3
6. 20-24 „	13.8	12.9

The protein consumptions recorded for Group 6 varied in the different periods according to the relative amounts of mash and grain consumed. The protein consumption of 14.5 per cent in period 1 was slightly lower than that consumed by Group 5 but in period 2A the figures were reversed. During periods 2B, 3 and 4 the protein consumptions of 14.8 per cent, 14.4 per cent and 13.9 per cent were higher than those recorded for Group 5 but in periods 5 and 6 the percentages of protein consumed were lower than in the corresponding periods for Group 5.

*Mortality.*—The percentage mortality from day-old to 24 weeks was 9.4 in Group 5 and 16.7 in Group 6. The birds in Group 5 invariably looked and handled much better than those in Group 6. During the early stages of the experiment, the birds in Group 6 looked somewhat dejected and many had loose feathers and drooping wings, whereas such symptoms were rare in Group 5.

*Egg production.*—Table XVII gives details in regard to the age at first egg, the percentage of birds laying and the average egg production and size at 24 weeks for each breed in the two groups.

TABLE XVII

*Egg production*

Group	5			6		
Breed	White Leg-horn	Rhode Island Red	Desi	White Leg-horn	Rhode Island Red	Desi
Age at first egg (days)	136	136	131	167	188	157
Percentage of birds laying at 24 weeks	57.9	20.0	63.6	7.7	<i>Nil</i>	5.0
Average number of eggs to 24 weeks	2.9	3.9	4.8	0.1	<i>Nil</i>	0.5
Average egg weight (oz.)	1.48	1.48	1.25	1.50	..	1.23

In Group 5 the first eggs were produced by the White Leghorns, Rhode Island Reds and *desis* at 136, 136 and 131 days respectively. In Group 6, the corresponding figures were 167, 188 and 157 days. In Group 5, 57.9 per cent of the White Leghorns, 20.0 per cent of the Rhode Island Reds and 63.6 per cent of the *desis* had started to lay by 24 weeks. In Group 6, the corresponding percentages for the Leghorns, Rhode Island Reds

and *desis* were 7.7, 0 and 5.0. At 24 weeks of age, the average egg production per bird in Group 5 was 2.9 for the Leghorns, 3.9 for the Rhode Island Reds and 4.8 for the *desis*. The corresponding figures for Group 6 were 0.1, 0 and 0.5.

*Conclusions.*—(1) Satisfactory results were obtained by feeding chicks on a basal ration *plus* separated milk only to drink from 0-6 weeks and separated milk and water to drink from 6 weeks onwards.

(2) The basal ration *plus* soya bean meal and salt did not give as good results as the basal ration *plus* milk from 0-6 weeks and milk and water to drink from 6 weeks onwards.

#### Experiment 4

The results obtained with ground soya beans in Experiment 3, though not so satisfactory as those obtained with separated milk, were considered sufficiently encouraging to warrant further trials with ground soya beans. In view of the fact that the early stages of growth are the most critical, both in regard to growth and mortality, it was decided to give all the birds a good start by feeding separated milk from 0-6 weeks. From six weeks onwards one group was fed the standard basal ration *plus* separated milk and water in separate containers and the other group on the basal ration *plus* ground soya bean meal and salt.

*Stock.*—A total of 168 day-old chicks, hatched in March, 1939, were wing-banded and divided into two similar groups as follows :—

Breed	Number of chicks	
	Group 7	Group 8
White Leghorn	14	15
Rhode Island Red	27	26
<i>Desi</i>	44	42
Total	85	83

*Feeding.*—Both the groups were fed on the standard basal ration *plus* separated milk to drink during the first six weeks. From 6 weeks onwards, Group 7 was fed the basal ration *plus* separated milk and water in separate containers; Group 8 was fed 81.5 per cent basal mash *plus* 18 per cent ground soya bean meal *plus* 0.5 per cent common salt and water to drink. The methods of feeding and management were identical with those adopted in the previous experiments but in this instance the cockerels were removed at 8 weeks.

*Growth results.*—Table XVIII gives a statement of the average weights of the cockerels and pullets of each breed at different stages from 0-24 weeks,

TABLE XVIII

*Average weights in ounces for each breed*

Breed	White Leghorn				Rhode Island Red				Desi			
	7		8		7		8		7		8	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day old	1.41	1.34	1.35	1.39	1.60	1.54	1.54	1.60	0.99	0.96	0.95	0.98
3 weeks	5.9	5.2	5.3	5.3	5.8	5.8	6.2	5.9	8.9	8.4	8.8	8.6
6 "	13.5	12.5	13.0	11.7	14.4	13.3	15.9	13.5	9.3	7.0	9.2	7.5
7 "	16.4	15.4	14.8	13.8	17.4	16.2	18.9	16.7	11.7	8.7	11.2	9.2
8 "	20.3	18.7	18.0	16.0	21.3	19.5	20.8	19.2	14.5	11.2	13.5	11.7
10 "		22.5		18.0		24.4		21.1		14.3		13.1
12 "		29.2		23.0		30.3		26.2		19.3		17.3
14 "		37.7		28.5		37.5		34.4		24.4		20.9
16 "		40.7		33.5		45.1		39.1		29.3		24.7
18 "		45.7		37.3		52.9		45.5		32.8		27.4
20 "		47.7		41.7		57.8		51.3		36.8		31.6
22 "		51.0		43.0		63.5		53.8		38.9		33.2
24 "		55.5		47.5		70.2		59.1		41.5		35.8



During the period 0-6 weeks, which may be looked upon as pre-experimental, both groups when fed on the basal ration *plus* separated milk made very similar gains in weights during each period of 3 weeks. At 6 weeks, when the experiment proper started, the weights of the males and females for each breed in Group 7 agreed very closely with the weights of the corresponding birds in Group 8. From 6 weeks onwards the birds in Group 7, fed on separated milk and water, made better gains in weight than the birds receiving the soya bean meal *plus* salt. At 12 weeks and at all subsequent weighings the pullets of all the three breeds in Group 7 were significantly heavier than the corresponding birds in Group 8. At 24 weeks the weights of the pullets in Group 7 were White Leghorns 55.5 oz., Rhode Island Reds 70.2 oz. and *desis* 41.5 oz. The corresponding weights of the White Leghorns, Rhode Island Reds and *desis* in Group 8 were 47.5, 59.1 and 35.8 oz. respectively.

*Food consumption.*—Table XIX gives the average food consumption and food utilization per bird for each group during the pre-experimental period 1 (0-6 weeks), experimental period 2 (6-8 weeks) and for each subsequent period of four weeks.

TABLE XIX

*Food consumption*

Period	Average food consumption per bird (lb.)		Food consumed in lb. per pound live weight gain	
	Group 7	Group 8	Group 7	Group 8
1. 0-6 weeks	2.28	2.26	3.86	3.84
2. 6-8 "	1.19	1.26	3.81	4.77
3. 8-12 "	2.75	2.46	4.62	6.18
4. 12-16 "	3.82	3.03	5.01	5.27
5. 16-20 "	4.87	4.69	8.25	8.41
6. 20-24 "	4.68	3.76	9.74	10.56

The food consumptions by both groups during the pre-experimental period were very similar. The efficiency of food utilization was also practically identical in both groups during period 1. Group 7, except in period 2, consumed slightly more food per bird in each of the subsequent periods. During periods 2 and 3, Group 7 consumed appreciably less food per pound of live weight gain than Group 8 but in the other periods there were no significant differences in the efficiency of food utilization.



*Protein consumption.*—Table XX gives details of the total protein percentages consumed during the different periods of the experiment.

TABLE XX

*Protein consumption*

Period	Percentage of protein	
	Group 7	Group 8
1. 0-4 weeks	14·2	14·1
2A. 4-6 „	15·9	16·3
2B. 6-8 „	15·4	14·5
3. 8-12 „	13·6	13·7
4. 12-16 „	14·0	13·0
5. 16-20 „	14·2	12·7
6. 20-24 „	14·5	12·3

During the pre-experimental periods 1 and 2A, the percentages of protein consumed by the two groups were very similar. Group 7 consumed a higher percentage of protein during period 2B than Group 8 but the figures recorded were similar in period 3. The percentages of protein consumed by Group 7 during periods 4, 5 and 6 were higher than those consumed in the corresponding periods by Group 8.

*Mortality.*—The mortality in the pre-experimental period was 7·6 per cent in Group 7 and 9·7 per cent in Group 8. During the experiment proper, from 6-24 weeks, there were no deaths in Group 7 and 3 deaths, representing 3·6 per cent, in Group 8. The general health of the birds in both groups was satisfactory but throughout the experiment the birds on the milk ration handled better and had a more alert and better bred appearance than those on the soya bean ration.

*Egg production.*—Owing to lack of trapnesting facilities, it was not possible to keep separate egg production records of the different breeds in the two groups. The first eggs from Groups 7 and 8 were obtained at 140 and 159 days respectively.

*Conclusions.*—(1) The basal ration *plus* separated milk to drink, as in former experiments, gave satisfactory results from 0-6 weeks.

(2) From 6 weeks onwards the basal ration supplemented by milk and water to drink gave better results than the basal ration supplemented by soya bean meal and salt.

## Experiment 5

The results obtained with ground soya beans in Experiments 3 and 4, though not so satisfactory as those obtained with separated milk, were considered sufficiently good to warrant a further experiment along similar lines to those adopted in Experiment 4. In order to give the birds a good start, all those used in Experiment 5 were fed on cereals *plus* milk from 0-6 weeks. The birds were all weighed individually at six weeks and divided into two comparable groups. From 6-20 weeks one group was fed on the cereal ration *plus* separated milk and water in separate containers and the other group on the cereals *plus* ground soya beans *plus* salt.

*Stock.*—A total of 145 six-week old chicks, hatched in April, 1939 were divided up into two comparable groups in regard to weight and sex as follows :—

Breed	Number of chicks	
	Group 9	Group 10
White Leghorn	23	23
Rhode Island Red	9	8
<i>Desi</i>	42	40
Total	74	71

*Feeding.*—The methods of feeding and management were identical with those used in Experiment 4. Group 9 was fed on the standard diet *plus* separated milk and water in separate containers and Group 10 received the standard ration *plus* 18 per cent soya bean meal and 0.5 per cent salt in the mash. The cockerels were removed at 10 weeks and the experiment terminated at 20 weeks owing to lack of accommodation.

*Growth results.*—Table XXI gives details of the average weights of the birds of each breed at intervals of 2 weeks from 6-20 weeks.

The average weights of the milk-fed birds became progressively greater at each weighing than the corresponding birds on the soya bean ration. At 10 weeks and at each subsequent weighing, the birds of all the three breeds on the milk ration were significantly heavier than the corresponding birds on the soya bean ration. At 20 weeks, the average weights of the pullets in Group 9 were White Leghorns 47.7 oz., Rhode Island Reds 53.6 oz. and *desis* 39.6 oz. The corresponding weights of the White Leghorns, Rhode Island Reds and *desis* in Group 10 were 33.5, 41.0 and 29.4 oz. respectively.

*Food consumption.*—Table XXII gives the average food consumption and food utilization per bird for each group during period 2 (6-8 weeks) and each subsequent period of 4 weeks.

TABLE XXI

*Average weights in ounces for each breed*

Breed	White Leghorn				Rhode Island Red				Desi			
	9		10		9		10		9		10	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
6 weeks	11.8	10.3	11.7	10.1	12.3	8.7	12.3	9.0	7.9	7.2	7.7	7.2
8 "	16.9	14.8	15.0	12.6	20.2	13.3	16.4	12.2	12.7	11.2	10.2	10.1
10 "	24.3	20.1	20.0	15.0	27.2	18.5	21.0	16.0	17.5	13.2	12.5	12.6
12 "		24.7		18.1		23.5		20.3		19.6		15.4
14 "		31.4		23.2		30.5		26.4		25.6		19.8
16 "		36.7		27.5		38.3		32.3		30.6		22.5
18 "		41.2		29.5		44.3		36.3		34.6		25.7
20 "		47.7		33.5		53.6		41.0		39.6		29.4

TABLE XXII

*Food consumption*

Period	Average food consumption per bird (lb.)		Food consumption in lb. per pound live weight gain	
	Group 9	Group 10	Group 9	Group 10
2. 6-8 weeks	1.37	1.12	4.55	5.85
3. 8-12 "	3.48	2.59	5.54	7.32
4. 12-16 "	3.89	3.60	5.76	6.33
5. 16-20 "	4.11	3.05	6.03	7.55

The average food consumption figures per bird in Group 9 for each period were greater than those in the corresponding periods in Group 10. During each period Group 9 made better utilization of the food consumed than Group 10.

*Protein consumption.*—Table XXIII gives details of the total protein percentages consumed during the pre-experimental and experimental periods.

TABLE XXIII

*Protein consumption*

Period	Percentage of protein	
	Group 9	Group 10
1. 0-6 weeks	16.2	16.2
2. 6-8 "	14.6	14.6
3. 8-12 "	14.8	14.2
4. 12-16 "	14.1	13.2
5. 16-20 "	14.5	12.1

During the pre-experimental period, when all the birds were run in one group, the total percentage protein in the food consumed was 16.2. During periods 2 and 3, both groups consumed fairly similar percentages of protein but, in periods 4 and 5, Group 9 consumed appreciably higher percentages of protein than Group 10.

*Mortality.*—The mortality in the pre-experimental period (0-6 weeks) was 8.2 per cent. During the experiment proper there were no deaths in Groups 9, and 2, representing 3.0 per cent, in Group 10. As in the previous experiments the general health of the birds, as judged by feathering, handling and general alertness, was slightly superior in the milk-fed group.

*Conclusions.*—(1) The basal ration *plus* separated milk to drink from 0-6 weeks and separated milk and water from 6-20 weeks gave satisfactory results.

(2) From 6 weeks onwards the basal ration supplemented by milk and water to drink gave better results than the basal ration *plus* soya bean meal and salt.

#### GENERAL DISCUSSION

A ration consisting of cereals supplemented by adequate amounts of green food and calcium proved very unsatisfactory for rearing chickens from day-old onward. Birds on this diet grew very slowly, were unthrifty in appearance, suffered an excessively high mortality and made poor utilization of the food consumed. Whilst fed on this diet, the birds had depraved appetite. The birds on *post mortem* examination were very much emaciated and the gizzards had lesions suggesting that the diet was deficient in the gizzard-promoting factor. Almquist and Stockstad [1937] report that the best practical sources of the gizzard factor are green food and wheat bran; but under the conditions of this experiment both these foods, though fed in liberal amounts, failed to prevent the occurrence of the typical lesions produced by feeding a ration deficient in the gizzard factor. However, the conditions of the experiment were different from those of Almquist, who obtained average growth on the diet, which was normal except for a deficiency in the gizzard factor. Milk appears to be a good source of this factor, for two birds from the water group which were subsequently fed on milk for a period of 14 days had normal gizzards at *post mortem*.

In all the five experiments, satisfactory results were obtained with the basal diet supplemented with separated milk only to drink during the early stages of growth and milk and water in the later stages. In Experiment 1, milk only to drink was given for the first 10 weeks but in the later experiments milk and water was fed from 6 weeks onwards.

It is obviously impossible to compare the growth results obtained in experiments carried out at different periods of the year, for climatic variations are known to influence the rate of growth [Kempster and Parker, 1936; Upp and Thompson, 1927]. The influence of climate can be seen in the falling off in the growth rate as the weather grew hotter, for at 6 weeks of age the birds hatched in January were considerably heavier than corresponding birds, hatched in April, reared on the same diet.

Though no conclusions can be drawn in regard to the optimum time to feed milk only to drink, the results with milk only to drink from 0-6 weeks and milk and water from 6 weeks onwards, are quite satisfactory, for the weights of the Leghorns and the Rhode Island Reds at all stages compare very favourably with published standards [Card and Kirkpatrick, 1918; Charles and Knandel, 1928; Kempster and Parker, 1936]. The feeding of milk and water instead of milk from 6 weeks onwards can also be recommended, for the birds fed in this way consumed large quantities of water with a corresponding saving in milk. The mortality from day-old onward, among the birds given milk only to drink during the initial stages, which ranged from 4.8 per cent



in Experiment 1 to a maximum of 9.4 per cent in Experiment 3, is very satisfactory. The majority of the deaths occurred in period 1 (0-4 weeks) and these were mainly amongst weaklings due to errors in incubation or constitutional defects. The food consumption and food utilization figures also compare favourably with published figures [Card and Kirkpatrick, 1918; Charles and Knandel, 1928; Kempster and Parker, 1936].

The results obtained in Experiment 2 show that it is advisable to feed milk only to drink instead of milk and water during the early stages of growth. During the early stages the group fed on milk and water to drink from day-old onward grew more slowly, had an appreciably higher mortality and consumed more food per unit of gain than the milk supplemented group. Though the milk and water group from day-old onward grew faster during the later stages, when both groups were fed on the same diet, than the birds fed on milk only to drink during the early stages, the birds were lower in weight at all stages and took longer to come into production. Further, the feeding of milk and water from day-old onward did not result in any saving in milk, for over the whole experiment the birds consumed more milk than the group receiving milk only to drink from 0-6 weeks.

In Experiment 3, the basal ration supplemented with ground soya bean meal and salt did not give as good results as the milk ration and the birds on the soya bean meal were significantly lighter at all stages from 4 weeks onwards. During the first 8 weeks the birds on the soya bean ration had a much poorer health record, a higher mortality rate and made poorer utilization of the food consumed. From 8 weeks onwards the food utilization figures in the soya bean meal group were very similar to those obtained in the milk group. The improved health record and the low mortality rate in the soya bean meal group during the later stages of growth suggested that soya bean meal might be used more successfully if the birds were fed initially on a milk diet and fed soya bean meal during the later stages.

In Experiments 4 and 5, all the birds were reared on the basal ration *plus* milk to drink from 0-6 weeks. From 6 weeks onwards, half the birds in each group were fed the basal ration *plus* separated milk and water to drink and the other half the soya bean meal *plus* salt ration. In both the experiments, the rate of growth of the birds on the soya bean ration was significantly lower than that of the birds on the milk and water ration. The efficiency of food utilization was lowered and egg production retarded in the soya bean groups. However, the health record was comparatively good and the mortality, though slightly higher than that of the milk group, was low.

The better results obtained with milk cannot be attributed to a higher protein level, for in Experiment 3 in periods 1, 2B, 3 and 4 the percentages of protein consumed by the soya bean meal group were higher than during the corresponding periods in the milk group. Similarly, in Experiments 4 and 5, the soya bean meal groups in certain periods consumed approximately the same percentages of protein as the milk groups.

The good growth results obtained with milk cannot be attributed solely to the excellence of its proteins, for milk also contains other valuable growth-promoting factors such as vitamins and minerals. Similarly, the poorer growth results obtained with soya bean meal cannot be attributed solely to

the poor quality of its proteins, for it is very possible that accessory food factors, such as vitamin B<sub>2</sub>, might have been at a sub-optimum level for growth. Further, as experiments at Illinois [Lippincott, 1936] have demonstrated that extracted soya bean meal gives better results than ground soya bean meal and, as it is well established that chicks make poor utilization of rations containing considerable amounts of fat, it is probable that the level of fat in the ground soya beans may have had a depressing effect on the rate of growth. As the unpublished data obtained from previous experiments carried out by the author at the Rowett Research Institute, Aberdeen, had shown that extracted soya bean meal supplemented by salt gave results somewhat similar to those obtained with separated milk, it is possible that better results might be obtained by using soya beans that have been subjected to a suitable process of oil extraction. Hayward and his co-workers [1937] found that extracted soya bean meal subjected to heat gives much better results than unheated soya bean meal. As separated milk is often an expensive article of diet, it is proposed to investigate these factors more fully in a further series of experiments.

Though separated milk was the sole milk product used, experiments by other workers [Card, 1926; Kempster and Parker, 1936; Swift, Black and Voris, 1931] demonstrate that whole milk, separated milk, buttermilk, dried skim milk and dried buttermilk have approximately the same nutritive value, when fed at similar protein levels.

The results with soya bean meal supplements, though not so satisfactory as those with milk, should not preclude the use of soya bean meal when milk is difficult to obtain or very expensive, for additions of soya bean meal and salt very materially improve the nutritive value of cereal rations. However, from the results obtained in Experiments 3, 4 and 5 it appears advisable to give milk during the early stages of growth.

The optimum protein levels in the ration of growing chicks have been investigated by a number of workers. Heuser and Norris [1930] conclude that chick rations should contain at least 20 per cent protein. Swift and his co-workers [1931] state that the best growth results are obtained with rations containing over 20 per cent protein. Hammond and his co-workers [1938] conclude that optimum growth in young chickens is obtained by feeding rations containing 21 per cent protein. In all the 5 experiments under review, where milk was used as the sole protein-rich food, the rate of growth of the Leghorns and Rhode Island Reds compares favourably with standard figures, even though the protein levels at all periods were very much lower than 20 per cent. The percentages of protein consumed by the different groups show a considerable similarity, the consumption being high from 0-8 weeks, low from 8-16 weeks and high from 16-24 weeks. In Groups 4 and 5, however, the protein consumption remained fairly steady throughout. This was probably due to the fact that the milk turned sour in the feeding vessels and was consequently more readily consumed than sweet milk. The average protein percentage figures in the food consumed for all 5 milk groups were.—Period 1 (0-4 weeks) 14.6, period 2 (4-8 weeks) 14.8, period 3 (8-12 weeks) 13.2, period 4 (12-16 weeks) 13.3, period 5 (16-20 weeks) 14.1 and period 6 (20-24 weeks) 14.3. Though it is quite possible that slightly better growth

might have been obtained with higher protein levels, the results obtained as regards rate of growth, food utilization, general health and mortality were satisfactory. The data collected suggest that the protein requirements for growth, when milk is used as the protein supplement, are considerably lower than the accepted standards.

#### GENERAL CONCLUSIONS

(1) The feeding of chicks on a mixed cereal diet, although supplemented with liberal amounts of green food and calcium, results in very poor growth, excessive mortality and poor food utilization.

(2) A ration containing liberal amounts of wheat bran and green food failed to prevent the occurrence of gizzard lesions, which appeared typical of those produced by diets deficient in the gizzard factor.

(3) During the early stages of growth (up to 6 weeks), a ration of cereals supplemented by liberal amounts of green food and calcium along with separated milk to drink proved adequate.

(4) During the early stages of growth the feeding of milk and water in separate containers gave substantially poorer results than when milk alone was provided as a drink.

(5) From 6 weeks onwards, the feeding of milk and water in separate containers gave satisfactory results as regards growth etc. and resulted in a considerable saving in cost.

(6) The nutritive value of cereal rations is greatly improved by a supplement of soya bean meal *plus* salt.

(7) Soya bean meal *plus* salt proved inferior to milk as a supplementary feed for chicks up to 6 weeks of age and to milk and water from 6 weeks onwards.

(8) The feeding of cereals *plus* separated milk to drink from 0-6 weeks and cereals *plus* soya bean meal and salt from 6 weeks onwards gives better results than cereals *plus* soya bean meal and salt from day-old onward. If no milk is available for chicks less than 6 weeks old, soya bean meal and salt can be used with fair success.

(9) The protein requirements for young chicks during early life are considerably lower than the accepted standard of 20 per cent when the protein supplement is provided in the form of milk.

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# OBSERVATIONS ON *BABESIA FOLIATA* N. SP. FROM A SHEEP\*

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(With Plate XVIII)

**PIROPLASMOSIS** is a very wide-spread disease of sheep and goats from which India is by no means free. Wenyon [1926] recognises three species of *Babesia* infecting sheep and goats, of which two, viz. *Babesia sergenti* and *Babesia motasi*, are the only ones recorded in this country. *Babesia sergenti* was encountered for the first time in India in the blood of a goat under rinderpest experiment at the Imperial Veterinary Research Institute, Mukteswar [1932], while Achar and Srikantiah [1934] have, under similar experimental conditions, observed the occurrence of *Babesia motasi* in the blood of sheep in Mysore. Sarwar [1935] described *Piroplasma taylori* from a goat in the Punjab. This organism was originally considered by Bhatia [1936] to belong to the genus *Theileria* but subsequently, after having examined the preparations of Sarwar, he [Bhatia, 1938] concluded it to belong to the genus *Babesia*. During the course of routine examination of blood smears of animals of this Institute the writers found a species of *Babesia* in the erythrocytes of a sheep which was being used in an experimental test with blackquarter vaccine. When compared with other ovine species of *Babesia*, this parasite was found to differ from them in many respects. In this article, therefore, it is described as *Babesia foliata* n. sp., the specific name being suggested by its general appearance, which is similar to that of a leaf.

## OBSERVATIONS ON THE MORPHOLOGY OF *BABESIA FOLIATA* N. SP.

The parasite, as mentioned above, has a leaf-shaped outline. The broad pole is rounded and is the widest part of the parasite. The same breadth is maintained for about two-thirds of the length while the superior third gradually tapers into a fine point (Plate XVIII, figs. 1 and 12). The majority of the parasites occur in pairs, there being a cytoplasmic connection between the pairs at the narrow pole, and usually they occupy the centre of the erythrocyte. Out of a small count of a hundred and twenty parasitised corpuscles, it was found that the organism was placed centrally in 82.5 per cent and

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peripherally in 17·5 per cent of the infected cells. The distribution of the angle between the pairs is indicated in Table I.

TABLE I

Angle	Position of the parasite in the erythrocyte	
	Central	Peripheral
180°	per cent 31	per cent 10
Obtuse	25	40
90°	29	30
Acute	9	20
Parallel	6	Nil

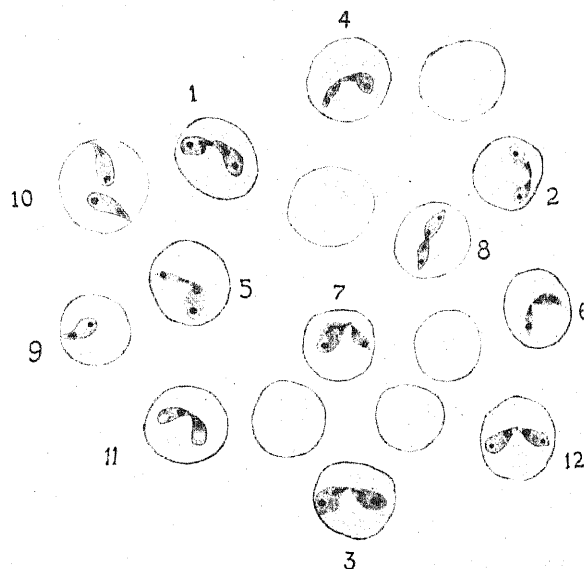
In cases where the parasites were found to be situated parallel to each other, no cytoplasmic connection between the pairs could be seen at the narrow pole. Occasionally, when forming an obtuse angle the parasites were so far away from each other that this cytoplasmic connection was very faint. Cells containing a single parasite were rarely seen in our preparations. Such rare forms were chiefly amoeboid in shape and showed signs of nuclear division. The stages of division of this parasite did not appear to differ in any way from those of *B. bigemina* described by Nuttall and Graham-Smith [1908], for which reason it has been considered unnecessary to describe them here. In amoeboid forms, as well as in the paired individuals, a vacuole was often seen occupying a central position. The size of the parasite varied from 2·065-4·13 $\mu$  in length and 0·5-1·5 $\mu$  in breadth. No hypertrophy was ever noticed in the infected corpuscles. The nuclear chromatin, as revealed by Feulgen's reaction, was found to be arranged in granules just below the stained nuclear membrane and to occupy the narrow pole of the organism. The central karyosome also gave a positive reaction with Feulgen's test (Plate XVIII, fig. 11). In Giemsa preparations a granule giving a deeply staining reaction similar to that of the nucleus was frequently seen at the broad pole of the organism, but, when put to Feulgen's test for thymonucleic acid, it was completely hydrolysed, suggesting that it has no relation with the nucleus of the parasite. On counterstaining with Heidenhain's iron-alum haematoxylin after Feulgen, however, this deeply staining granule, as mentioned above, reappeared at the broad pole of the parasite (Plate XVIII, fig. 12).

This parasite when compared with *Babesia motasi* Wenyon [1926] differed in its measurement, in the angle between the pairs and in the absence of hypertrophy of the parasitised erythrocyte. When compared with *Babesia ovis*



*Babesia foliata* n. sp.

[ All figures are *camera lucida* drawings of smears preparations stained with Giemsa (unless otherwise stated).  $\times 2300$  ]



FIGS. 1-3. Pairs showing typical leaf-like outline of the parasite.

FIGS. 4-7. One individual in the pair presenting an edge-on view.

FIG. 8. Individuals of a pair situated at an angle of  $180^\circ$ .

FIG. 9. A single parasite which appears to have just entered a corpuscle.

FIG. 10. Parasites about to leave the corpuscles.

FIG. 11. From wet smear fixed in Bouin and Duboscq fluid and stained with Feulgen and counterstained with light-green. Note the arrangement of chromatin in the nucleus at the narrow pole and the hydrolysed vacuolated area at the broad pole of the parasite.

FIG. 12. Fixed as above and stained with Feulgen and counterstained with Heidenhain's iron-alum-haematoxylin. Note the hydrolysed area which has taken a deep haematoxylin stain.

N.B.—The arrangement of nuclear chromatin in figures 11 and 12 can be well seen with the aid of a magnifying glass.



Starcovici [1893], this parasite differed by occurring almost always in pairs, producing a heavier infection, and also in its location in the majority of cases in the centre of the cell. The parasite also differs from *B. taylari* Sarwar [1935] in the absence of extra-cellular multiplication, its ovoid and rounded body, the hypertrophy of the infected corpuscle, and multiple infection of the cell. There would appear to be no similarity between this parasite and *Babesia sergenti* Wenyon [1926], as the latter is rounded or bacillary in form and is non-pathogenic. Moreover, according to Thomson and Hall [1933], *B. sergenti* is placed under the genus *Theileria*. The accompanying table compares the species of *Babesia* recorded from sheep and goats in respect of their shape and size, the angle between the pairs, their position in the corpuscle and the effect on the parasitised erythrocyte.

TABLE II

*Comparisons of the species of Babesia from sheep and goats*

Characters	<i>B. motasi</i> Wenyon (1926)	<i>B. ovis</i> Starcovici (1893)	<i>B. sergenti</i> Wenyon (1926)	<i>B. taylari</i> Sarwar (1935)	<i>B. foliata</i> n. sp.
Shape	Pear-shaped, singles, and pairs	Pear-shaped forms rarely seen. Singles majority round	Round and bacillary	Mostly ovoid and round; pear-shaped forms rarely seen. 1 to 16 parasites seen in one cell	Flattened, leaf-shaped in outline. Mostly in pairs. Rarely more than one pair seen in one cell
Size in microns	2.5-4.5 x 1.2-3.0	Never exceeds 2.0	?	2.0 x 1.0	2.065-4.18 x 0.1-1.5
Angle between the pairs	Acute	Obtuse	?	?	Majority obtuse
Position in the red blood corpuscles	Centre	Margin	Centre (?)	When central singles	Majority centre
Effect on the parasitised corpuscle	Hypertrophy	No change	No change (?)	Hypertrophy	No change
Host	Sheep and goats	Goats	Sheep and goats	Goats	Sheep

#### SYMPTOMS OBSERVED IN THE HOST

The infection in the sheep referred to above was heavy and was accompanied by a rise of the temperature ranging between 102.4-104.4°F. The general condition deteriorated daily. No haemoglobinuria was detected by applying the Benzidine test to the urine. Microscopically, there were considerable anaemic changes, which consisted of marked anisocytosis, slight polychromatia and marked increase in the punctate basophils. The red blood corpuscles towards the latter part of the disease showed an increase in the central halo and ultimately only the margins of the corpuscles were stained.

## TRANSMISSION

The transmission of the disease was successfully carried out by direct intravenous inoculation of blood from the natural case into two experimental sheep. Goats were also inoculated with the infected blood but they failed to react. The parasites were later found in the blood of the two sheep. In one, the parasite was seen on the third and fifth days, and in the other on the fourth and twenty-first days, after inoculation of infected blood. With the appearance of the parasite in the peripheral circulation the animals showed thermal reactions. These sheep also showed in their blood anaemic changes similar to those observed in the natural case. Subsequently, these changes disappeared and the animals recovered without the intervention of any drug.

## DIAGNOSIS

Systematic position. *Babesia foliata* n. sp. (Haemosporidiida, Babesiidae).

*Description*.—Flattened leaf-shaped in outline ; mostly seen in pairs, angle between the pairs, obtuse ; mostly situated in the centre of the erythrocyte ; produces no change in the infected corpuscle ; size,  $2.065-4.13\mu \times 0.5-1.5\mu$ .

*Habitat*. Erythrocytes of sheep.

*Locality*.—Mukteswar-Kumaun, U. P., India.

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# EXPERIMENTAL INFESTATION WITH *FISCHOEDERIUS ELONGATUS* IN A CALF AT MADRAS

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RAO and Ayyar [ 1932 ] have determined by infection experiments carried out in Madras that *Cercariae Indicae* XXIX Sewell, 1922 is the larval stage and the snail *Limnea leuteola* (Lamarck) an intermediate host, of the amphistome *Fischoederius elongatus*. An experiment was therefore performed to confirm these findings, as well as to find the clinical significance in an infection.

*Cercariae Indicae* XXIX Sewell, 1922 discharged by the snail *Limnea leuteola* (Lamarck) were allowed to encyst on blades of grass and a total of 250 encysted cercariae, collected within a week, were fed on the 7th April 1940 to a heifer calf aged about a year and three months. The calf used for the experiment was under observation for seven-and-a-half months prior to the date of infection and it was kept during the whole period in experimental sheds of the College Laboratory under conditions designed to prevent extraneous parasitic infection. Previous infection with any amphistomes was ruled out by a daily examination of the dung which was negative for their ova. The calf was destroyed on the 14th June 1940, nine weeks after the date of infection. At *post-mortem* ninety adult *Fischoederius elongatus* were collected from the wall of the rumen.

In this infection, producing 36 per cent adult amphistomes out of 250 encysted cercariae fed, during the development of the parasite within the host, there was no systemic disturbance met with. The temperature ranged from 100°F. to 102°F. touching 102°F. for only a couple of days. The dung was normal and the appetite good throughout. Though faecal examination made every day prior to destruction revealed no ova of amphistomes, eggs were detected mixed up with the contents of the rumen at *post-mortem*. It may, therefore, be said that eggs are discharged by these amphistomes by the ninth week after infection.

## ACKNOWLEDGEMENT

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# A STUDY ON GOAT SPLEEN TISSUE VACCINE AS AN IMMUNIZING AGENT AGAINST RINDERPEST

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(With one text-figure)

## INTRODUCTION

THE preparation of a single, safe and cheap product for the effective immunization of cattle and buffaloes against rinderpest has been engaging the attention of various workers for a long time past, and the use of goat spleen tissue preparations may be said to be the outcome of such work. Based on the results published by Kerr and Menon [1934] and Kerr [1935] on the use of goat spleen tissue for the control of the disease in Bengal, experiments were conducted at the Serum Institute, Madras, under laboratory conditions, with a view to study the adaptability of the same technique in the Madras province.

## METHOD OF REMOVAL OF SPLEEN

Healthy young goats are injected with 5 c.c. of rinderpest goat blood virus, and killed at the height of thermal reaction usually on the 4th or 5th day. As the normal temperature of goats in Madras is about 100° F. a temperature of 103° F. and above in the morning is considered to be a satisfactory reaction. The goat is bled for virus and the abdomen is then shaved and disinfected and the spleen removed under aseptic precautions.

## EXPERIMENTS CONDUCTED AT THE INSTITUTE

Spleen taken from goat No. 856 of the 300th passage of goat virus, Mukteswar strain (Fig. 1), was used. The spleen was cut into pieces weighing approximately one gramme each and put into small sterile test-tubes which were then sealed with cotton plugs dipped in boiling paraffin. The tubes were then packed in a case with damp cotton wool, and kept at room temperature which ranged from 83·8° to 86° F. (The temperature of the tube was less by nearly 3·5° F. than the temperature of the room and this difference in temperature was noticed for 10 days after which the temperature of the tube equalled the temperature of the room, since all the moisture had evaporated from the damp cotton wool by that time.)

## TEMPERATURE CHART

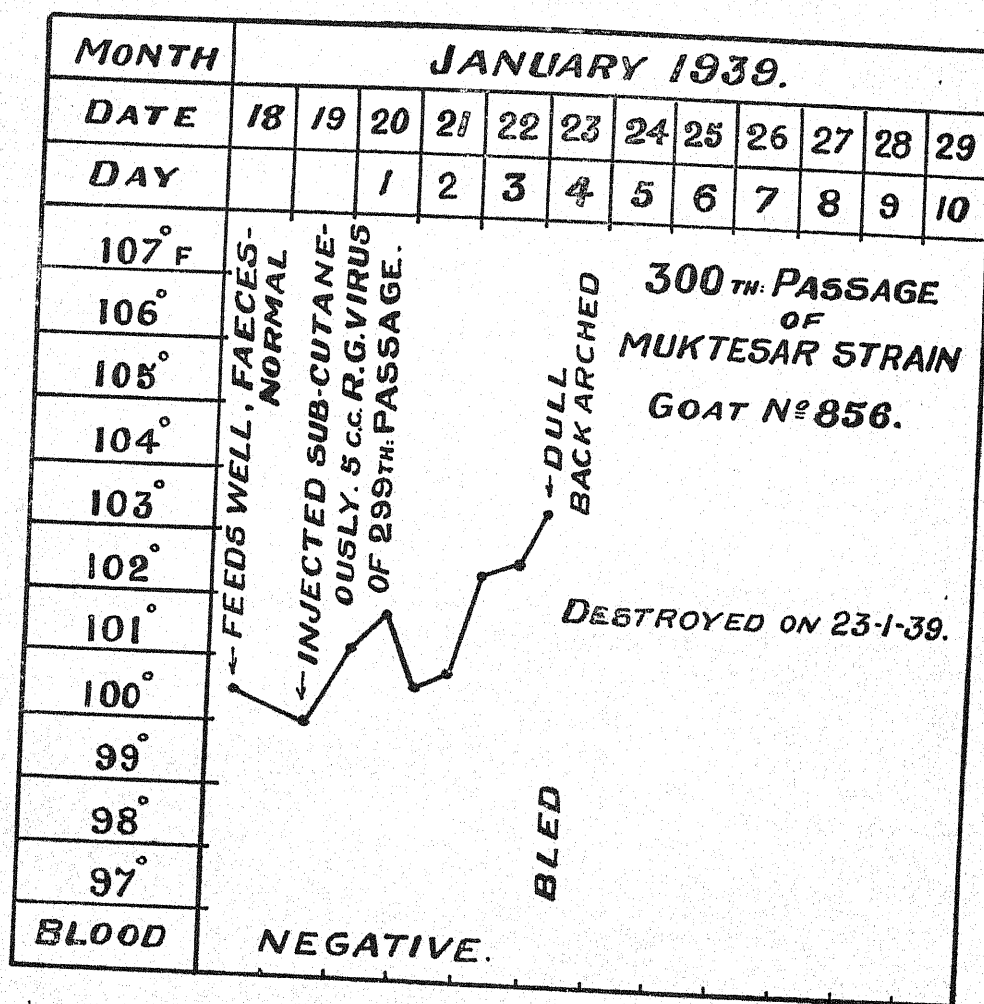


Fig. 1.

The experiments were commenced with one per cent emulsion of fresh spleen (one gramme of the tissue in 100 c.c. of 0.5 per cent saline solution) and were continued with emulsions of the same strength prepared from the spleen tissue stored at room temperature for one to ten days, the temperature of the room ranging from 83.8° to 86° F. In each case four buffalo-calves were injected, one pair receiving a dose of 1 c.c. each, while the other pair received a dose of 0.5 c.c. each, of the emulsion. Different doses (0.5 c.c. and 1 c.c.) were adopted to study if the dose given had any effect on the reaction produced.

No sub-inoculations were conducted in this series of experiments but all animals that survived were tested with bull virus.

## DETAILS OF REACTIONS

Forty-four buffalo-calves were used in all, in this experiment. For purposes of classification of reactions, they might be grouped into two batches :

*First batch.*—This batch consisted of twenty calves ten of which received a dose of 0·5 c.c. each and the remainder 1 c.c. each of the emulsion prepared from fresh spleen tissue or that stored at room temperature from 1 to 4 days. All the calves reacted severely and four of them died. They showed high temperature and diarrhoea and in some cases vesicles in the mouth were also observed. The dose, either 0·5 c.c. or 1 c.c., had no appreciable difference in the severity of reactions set up.

*Second batch.*—This batch consisted of twenty-four calves, twelve of which received a dose of 0·5 c.c. each and the remainder 1 c.c. each of the emulsion prepared from spleen tissue kept at room temperature for 5 to 10 days. None of the animals reacted to vaccination, but on retest with bull virus all of them reacted severely. Only one calf which received 1 c.c. of the emulsion stored at room temperature for nine days showed thermal disturbance and diarrhoea and died. Probably it was in the incubative stage of the disease at the time of inoculation. On retest with bull virus almost all the calves reacted severely and twelve died.

## SUMMARY OF REACTIONS

(1) Emulsion of fresh goat spleen tissue as well as emulsions prepared from spleen stored at room temperature for 1 to 4 days (one per cent emulsion in 0·5 per cent saline) produced uniformly severe rinderpest reactions in buffalo-calves in doses of 1 c.c. and 0·5 c.c. The animals that survived withstood the retest with bull virus and did not react.

(2) Emulsions prepared from spleen tissue stored at room temperature for 5 to 10 days did not produce any reaction and conferred no immunity as judged by the severe reactions on retest.

(3) The dose of either 0·5 c.c. or 1 c.c. made no appreciable difference, either in the severity of reactions produced or in the degree of immunity set up.

## EXPERIMENTS IN THE FIELD

Trials with this product were carried out in the field also. The sealed tubes containing one gramme pieces were sent in thermos jars packed with ice, with instructions to use the vaccine immediately on receipt. Each one gramme piece was ground gently in a sterile mortar with 100 c.c. of normal saline solution. The emulsion was then filtered through sterile gauze and the filtrate was used, the dose adopted being 1 c.c. per animal. From the reports received, the following observations are made :—

Six thousand two hundred and eight cattle and 910 buffaloes were so far vaccinated, out of which 5,669 cattle and 784 buffaloes could be observed for reactions. The vaccinations were performed both in clean and infected areas. The reactions may be classified under two headings—mild and severe. 45 per cent of the cattle and 34 per cent of the buffaloes had mild reactions. 22·5 per cent of the cattle and 46 per cent of the buffaloes had severe reactions.

There was a mortality of 0·5 per cent in the cattle and 2·5 per cent in the buffaloes. 32 per cent of the cattle and 20 per cent of the buffaloes showed no reaction to vaccination. In some cases, anti-rinderpest serum had to be used to check the severity of reaction.

#### IMMUNITY TEST

Immunity tests with rinderpest bull virus were conducted on a few vaccinated animals in selected areas a few months after the original vaccination and it was found that the immunity was solid.

#### DISCUSSION

It was found, both from experiments conducted in the laboratory and in the field, that the method of vaccination with an emulsion of one per cent strength of infected goat spleen, in normal saline, was not altogether satisfactory, as it produced severe reactions and even mortality in some cases. Though in Bengal very good results were reported with this method of vaccination by Kerr & Menon [1934] and Kerr [1935], yet in this province the experience was such that it was not possible to encourage this method. Many of the vaccinated animals had good reactions which were nearly as severe as those seen in goat blood virus alone method and quite alarming enough for the ryots. Many animals were reported to have lost their condition after vaccination, and in case of working animals which were put to work after vaccination, the reactions were found to be severe. It was also found necessary in some cases to give anti-rinderpest serum to modify the severity of reactions. In Bengal, this method was found to be efficient and safe to combat rinderpest outbreaks [Kerr & Menon, 1934], but later on, however, the experience as stated in the annual report of the Civil Veterinary Department, Bengal [1938-39] was that this method had not only caused severe reactions which proved fatal, but there was evidence of the introduction of infection in apparently healthy herds leading to outbreaks of rinderpest.

#### CONCLUSIONS

Rinderpest goat spleen tissue is potent for four days when stored at room temperature; the immunity conferred is satisfactory.

The reaction set up in the susceptible buffalo-calves is severe, a mortality of 20 per cent being observed in the calves when infected with potent goat spleen.

The application of this method of vaccination without serum in combating outbreaks of rinderpest is not suitable when the susceptibility of cattle and buffaloes is high, as it may produce reactions the nature of which sometimes creates panic among the ryots.

#### SUMMARY

Experiments were conducted at the Serum Institute, Madras, with saline emulsions of rinderpest goat spleen tissue stored at room temperature for varying periods. It was found that emulsion of fresh goat spleen tissue as



well as emulsions prepared from spleen tissue stored at room temperature for 1 to 4 days (strength of the emulsion being one per cent in 0·5 per cent saline) produced uniformly severe rinderpest reactions in buffalo-calves, in doses of 1 c.c. and 0·5 c.c. and conferred immunity. Emulsions prepared from spleen tissue stored at room temperature for 5 to 10 days did not produce any reaction and had no antigenic value. The dose of either 0·5 c.c. or 1 c.c. made no appreciable difference in the severity of reactions produced or in the degree of immunity set up. The adoption of this method in the field to combat outbreaks of rinderpest was not found to be quite safe in the Madras province as the reactions set up after vaccination were rather severe.

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*Experiments with goat spleen tissue vaccine (one per cent emulsion in 0.5 per cent. saline)*

Serial No.	Nature of the goat spleen tissue vaccine used	Dose	Number of calves used	Reactions	Reactions after retest with rinderpest bull virus	Remarks
1	Fresh vaccine	1 c.c.	2	Severe; one died	<i>Nil</i>	One died after retest due to emaciation
	Do.	$\frac{1}{2}$ c.c.	2	Severe; both died.	...	
2	Tissue kept in room temperature for one day	1 c.c.	2	Severe; one died	<i>Nil</i>	One died after retest due to emaciation
	Do.	$\frac{1}{2}$ c.c.	2	Severe; both died	...	Do.
3	Tissue kept in room temperature for two days	1 c.c.	2	One moderately severe and one non-reactor	<i>Nil</i>	
	Do.	$\frac{1}{2}$ c.c.	2	One severe and one mild	<i>Nil</i>	
4	Tissue kept in room temperature for three days	1 c.c.	2	One moderately severe and one non-reactor	No reactions in one and severe in the non-reactor	
	Do.	$\frac{1}{2}$ c.c.	2	Severe; one died	<i>Nil</i>	
5	Tissue kept in room temperature for four days	1 c.c.	2	One severe and the other non-reactor	No reactions in one and severe in the non-reactor which died	
	Do.	$\frac{1}{2}$ c.c.	2	Mild	<i>Nil</i>	
6	Tissue kept in room temperature for five days	1 c.c.	2	<i>Nil</i>	Severe; both died	
	Do.	$\frac{1}{2}$ c.c.	2	<i>Nil</i>	Severe; one died	
7	Tissue kept in room temperature for six days	1 c.c.	2	<i>Nil</i>	Severe; both died	
	Do.	$\frac{1}{2}$ c.c.	2	<i>Nil</i>	Do.	
8	Tissue kept in room temperature for seven days	1 c.c.	2	<i>Nil</i>	Severe	
	Do.	$\frac{1}{2}$ c.c.	2	<i>Nil</i>	Severe; one died	
9	Tissue kept in room temperature for eight days	1 c.c.	2	<i>Nil</i>	Mild in one and severe in the other which died	
	Do.	$\frac{1}{2}$ c.c.	2	<i>Nil</i>	Severe	
10	Tissue kept in room temperature for nine days	1 c.c.	2	<i>Nil</i> in one and severe in the other* which died	Severe	*Probably this calf was in the incubative stage of the disease at the time of inoculation
	Do.	$\frac{1}{2}$ c.c.	2	<i>Nil</i>	Severe	
11	Tissue kept in room temperature for ten days	1 c.c.	2	<i>Nil</i>	Severe and one died	
	Do.	$\frac{1}{2}$ c.c.	2	<i>Nil</i>	Severe and both died	

BREEDING COLOURATION OF *BARBUS (PUNTIUS)*  
*STIGMA* (CUV. & VAL.)\*

BY

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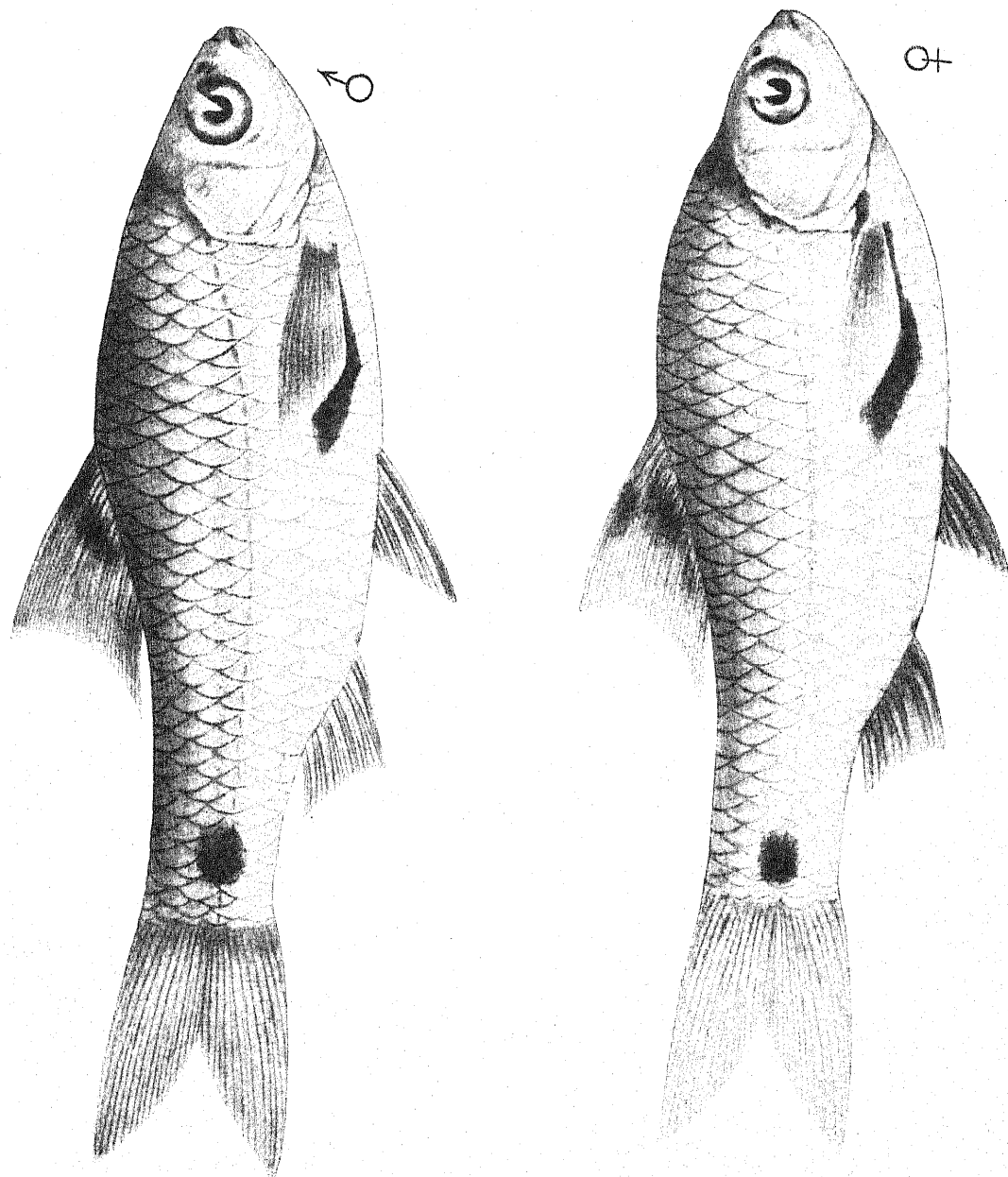
(Received for publication on May 3, 1941)

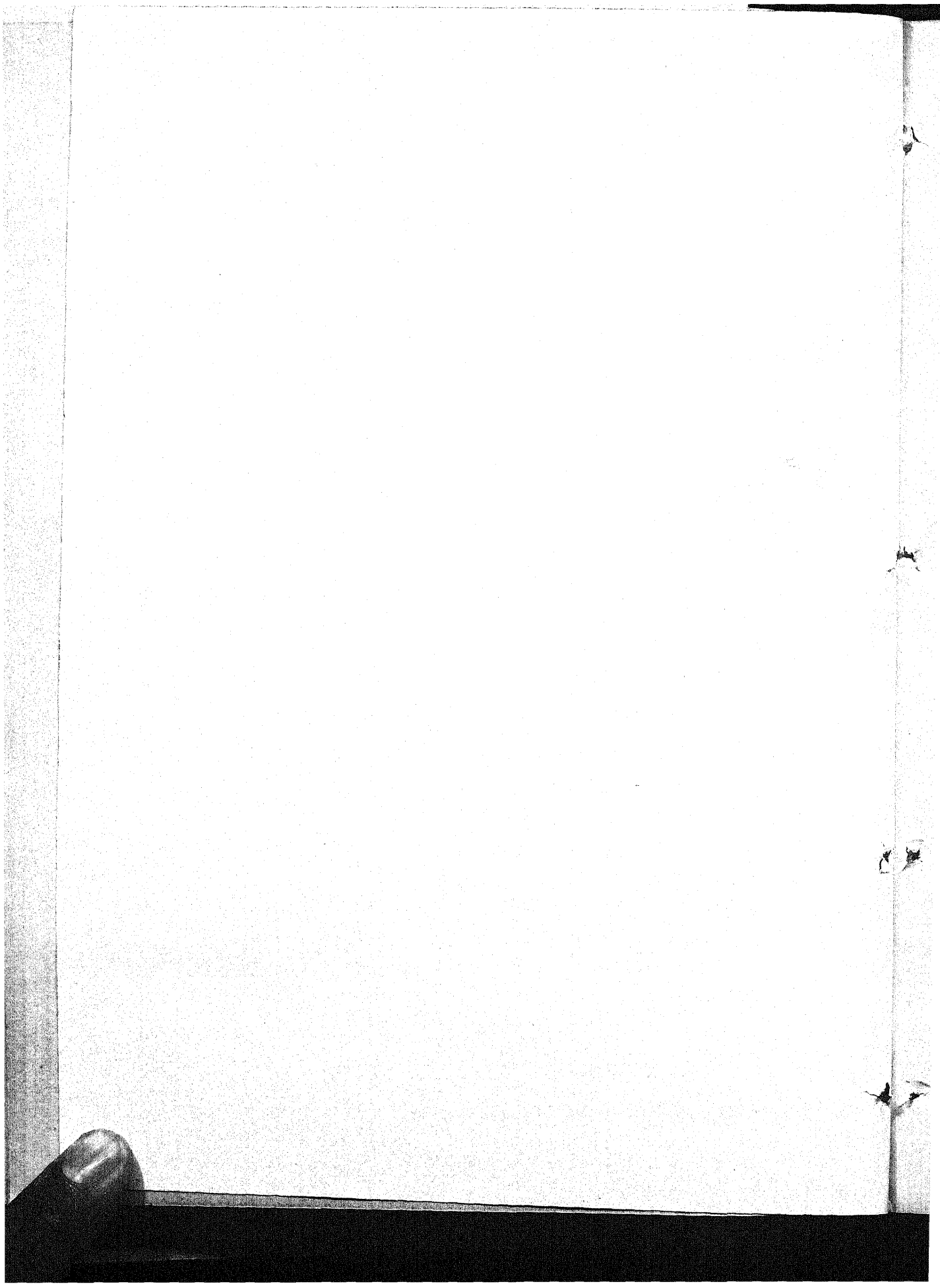
(With Plate XIX)

*BARBUS (PUNTIUS) STIGMA* is a small Carp Minnow commonly found in ponds, pools and ditches of Bengal; it usually grows to a size of 2 to 3 inches, but according to Day [1878], 'It attains at least 5 inches in length.' Day mentioned that 'As food it is bitter'. We have, however, found that the flesh of the fish is not bitter, but as the gall-bladder is got rid of by external pressure at the time of preparation of the fish, sometimes a certain amount of bile remains inside and this makes the fish bitter. During the dry season it is caught by the poorer people in large quantities by inexpensive methods of fishing [Hora, 1933]. The fish can easily be reared in small aquaria, but owing to its dull colouration it has not found favour with aquarists. In the course of our investigations on the life-histories of the freshwater fishes of Bengal, we noticed that during the breeding season, which lasts from April to July, both sexes develop a characteristic band of carmine red colour along the lateral line. In the male it is well defined and of a deeper colour and extends to the end of the caudal fin, while in the female it is more or less diffuse and only extends up to the end of the caudal peduncle. (Plate XIX). Usually the female develops this colouration only when fully ripe or immediately before it is ready to lay eggs, while in the male the colour band is developed with the ripening of germ cells. The body of a mature female is proportionately deeper than that of the male. Day (*loc. cit.*) also noted that the species is marked 'with a scarlet lateral band at some seasons', but evidently he did not realise its significance.

In several species of Carp Minnows the males assume brilliant colour during the breeding season [Innes, 1935] and during these periods sexes can

\*Chaudhuri [1916] has shown that *Barbus (Puntius) stigma* (Cuv. & Val.) is a synonym of *Barbus (Puntius) sophore* Hamilton. In view of the fact that Hamilton [1822] found this fish 'very common in ponds' of Bengal, we consider that Chaudhuri's views are probably correct, but as Day's *Fishes of India* and the volumes in his *Fauna of British India* series are still our standard works on Indian fishes we have retained the specific name *stigma* in Day's sense to facilitate reference.





be readily distinguished. The Indian species of *Barbus* in which this character has already been noticed are *B. (Puntius) chola* Ham., *B. (Puntius) conchoni* Ham., *B. (Puntius) gelius* Ham. and *B. (Puntius) terio* Ham. Misra [1938] described the colouration of preserved specimens of the male and the female of *B. (Puntius) malanampyx* Day, while Hora and Misra [1938], in describing the secondary sexual characters of *B. (Puntius) ticto* Ham., referred to the differences in the colour of the preserved male and female specimens. Hora, Misra and Malik [1939] have also referred to the differences in the colouration of the male and female specimens of *ticto* and *conchoni*.

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## SELECTED ARTICLES

### EXPERIMENTS ON THE ANTHELMINTIC ACTION OF PHENOTHIAZINE

BY

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(Reprinted from the *Veterinary Record*, Vol. 52, No. 36, September 7, 1940)

THE experiments described below were begun in October 1939, and formed part of the field trials which, as Dr. Taylor explains elsewhere in this symposium, were instituted by the Agricultural Research Council. They were planned with the advice and practical assistance of Professor Dalling, F. Blakemore, M.R.C.V.S., D.V.S.M., F. Day, F.R.C.V.S., and J. A. J. Venn, M.R.C.V.S., D.V.S.M. Professor Keilin, Director of the Molteno Institute, University of Cambridge, made the spectroscopic examinations of the blood of some of the horses. The egg and differential worm counts would not have been possible without the skilled help of H. E. Bowman. The phenothiazine used was supplied by the Agricultural Research Council and was therefore part of the stock which was also supplied to the other contributors to this symposium. Most of the contributors judged the effect of phenothiazine by egg counts and periodical weighings of the animals treated and the writer also followed this practice, except that he did a series of egg counts before the dose was given and took the average, in every instance, between the counts done on two different portions of the same sample of faeces, these two counts being done by different observers. These two counts on the same sample of faeces often differed very considerably, so that the precaution of taking the average of two was amply justified. It was unfortunately not possible to supplement the egg counts and weighings with autopsies, histological examinations of organs removed, especially of the kidney and the liver and by examinations of the blood (although the blood of some of the horses was examined), but these further examinations are being done on animals being used for additional experiments now in progress. The object of these additional experiments is to gather further information about (1) the relative efficiencies of copper sulphate alone, copper sulphate and nicotine, phenothiazine suspended in liquid, and tablets of phenothiazine; (2) the effect of phenothiazine on the host; (3) the greater or less effect of phenothiazine on particular species of nematodes. There is some evidence in the literature and in private communications to the writer, which suggests that phenothiazine acts most powerfully on nematodes living in the abomasum and in the lower parts of the bowel, and that it has less effect on nematodes living in the duodenum and the upper part of the small intestine. This evidence is not in any way conclusive, but it seems to the writer that this question should be investigated further. The study of it could well be combined with a study of (4) the effects, if any, of the environment of intestinal nematodes on phenothiazine and (5) the mode of action of phenothiazine, about which we know nothing at all. Taylor records in this

symposium that phenothiazine, in amounts far too small to have any anthelmintic action in a host animal, will inhibit the development of eggs and early larvae, although they do not affect the infective larvae. Such *in vitro* observations suggest a direct action of the drug. On the other hand, there is evidence which suggests that the action of phenothiazine is more pronounced in ruminant hosts, in which the drug has to pass through the rumen and other oesophageal chambers of these animals, than it is in omnivorous hosts such as the pig and the dog. It is possible that the intestinal contents of ruminants allow the drug freer access to the nematodes than the pastier faeces of omnivora do, but other factors probably operate as well. Swanson, Harwood and Connelly (1940) (*J. Amer. vet. med. Ass.* **96**, 333-338) found that phenothiazine was as effective as oil of chenopodium for the removal of ascarids from mature pigs and suggest that the ascaricidal principle of phenothiazine may be "activated by the substances excreted by those worms." They thought it was more effective when the infestations were heavy than when they were lighter. On the other hand, Singer and Baker (1940) (*Cornell Vet.* **30**, 375-382) found that phenothiazine was more effective against lighter nematode infestations of sheep than against heavier ones. Observations like these do, nevertheless, justify the question, which is also raised by other points in the literature: Is phenothiazine inactive as an anthelmintic until it is chemically changed in the gut, so that its action is greater on species living in the lower part of the alimentary canal? There is urgent need for the investigation of this and collateral questions. Associated with them is the problem of why it is necessary, as all workers agree that it is, to give so much phenothiazine, in order to produce an adequate anthelmintic effect, that a great deal of it is passed out again in the faeces apparently unchanged. This is a waste that might be avoided if we knew how phenothiazine acts on a nematode; we might then be able to use a modified form of it, or a derivative of it, which would be more potent and perhaps effective for all nematodes.

**TECHNIQUE.**—Differential worm counts were made by Taylor's technique (1934) (*Vet. Rec.* **14**, 474-475).

Egg counts were done by taking 3 grammes of faeces from the animal, either with the finger, or as they were passed, and emulsifying these in 45 c. c. water; 0.15 c. c. of this emulsion was then taken with a pipette and the eggs in this amount counted. Multiplication by 100 gave the number of eggs per gramme.

Wherever possible two counts, made by different observers (Lapage and Bowman), were made from different portions of each sample of faeces and the average of these was taken. Frequently these two counts differed widely. Sometimes one was negative, while the others showed as many as 700 e.p.g. or there were variations as wide as 7,500 and 200 e.p.g. in different portions of the same sample of faeces. It was evident, therefore, that the average of the two counts did not accurately represent the egg content of the faecal samples, but this kind of error is common to all the egg counts made and will not surprise helminthologists. In some experiments composite egg counts of mixed samples from individual animals were made, because it was not possible to do egg counts on each individual animal. Some of the disadvantages of composite egg counts are discussed below.

Swanson, Harwood and Connelly (1940) (*J. Amer. vet. med. Ass.* 96. 333-338) who studied the action of phenothiazine on *Ascaris* in pigs, used two kinds of phenothiazine, namely, "conditioned" phenothiazine, containing a dispersal agent to render it suitable for use as an insecticidal spray and "recrystallised" phenothiazine, which was more effective than the "conditioned" product. The writer is informed by Imperial Chemical Industries, who supplied to the Agricultural Research Council the phenothiazine used by the writer, that their phenothiazine, being a distilled product, can be regarded as being as pure as the recrystallised product used by Swanson, Harwood and Connelly.

## SHEEP AND LAMBS

### 1.—EXPERIMENT ON TWO LAMBS AND ONE EWE

These three animals were kept indoors and in the same box throughout the experiment. There was therefore the possibility that self-and cross-infestation could occur. Egg counts were done daily on all three animals for ten days before the phenothiazine was given and, with the exception of a few days, for three months after the dose. The ewe was not dosed and constituted a control.

On 2-11-39 the two lambs, called Marked and Unmarked respectively, were dosed with the tablets of phenothiazine first issued by Imperial Chemical Industries, namely, the 1 gramme tablets, each containing 1 gramme phenothiazine, six of these being enclosed in each gelatine capsule. Each tablet also contained some phenolphthalein and also some tartaric acid and sodium bicarbonate, the two latter ingredients being included to ensure the breakdown of the tablet in the stomach.

On 10-11-39 the Marked lamb, weighing 74 lb., was given four capsules containing a total dose of 24 grammes of phenothiazine (about 0.3 gramme per lb. body weight). On the same date the Unmarked lamb, weighing 89 lb., was also given a total dose of 24 grammes in four gelatine capsules (about 0.27 gramme per lb. body weight).

The effect of these doses on these two lambs was, in both cases, immediate. The day after the dose, the egg count of the Marked Lamb had dropped from a mean of 7,400 to a mean of 1,700. Three days after the dose the mean egg count was 200 e.p.g. and it remained at or near this figure until 56 days after the dose. An attempt was then made to remove the remaining few worms, which were producing a mean egg count at that time of about 200 e.p.g., by giving on 5-1-40, 7 grammes phenothiazine in the form of the same tablets as those that had been given before. This did not lower the egg count. On 10-1-40 a further six tablets (6 grammes) were given, but the egg count was still not appreciably affected. On 18-1-40 and 19-1-40, 12 and 13 days respectively after the second dose, successive doses of 6 grammes of the same tablets were given and the egg count dropped to a mean of 100 e.p.g. Successive doses of 6 grammes of the same tablets were repeated on 25-1-40 and 26-1-40 and the egg count was negative for the next three days, showing a mean of 100 e.p.g. again on the fourth day. Three successive daily doses of 6 grammes of the same tablets were then given on 31-1-40, 1-2-40 and 2-2-40 but, with the

exception of a negative egg count on the day after the last of these three doses, no lasting effect on the egg count was noted. No further doses were therefore given. The egg counts remained at a mean of 100 for a further 14 days after the last dose, when the egg counts were discontinued. The animal was however, kept in the box with the Unmarked Lamb until 8-4-40. On 7-4-40 and 8-4-40 its egg count was 0 and 100 respectively.

The history of the Unmarked Lamb was similar. After the initial dose of 0.27 gramme per lb. body weight (24 grammes) its egg count dropped from a mean of 3,850 e.p.g. to a mean of 550 e.p.g. It remained slightly higher than that of the Marked Lamb. On 6-1-40, 57 days after the initial dose, an attempt to remove its remaining worms was made by giving it in water as a drench 9 grammes of phenothiazine powder. Two days after this dose the egg count had dropped from a mean of 500 e.p.g. to 0. The next day (9-1-40) it had risen again to 50 e.p.g. and remained at this level till 15-1-40, when it was 200 e.p.g. On 17-1-40 it was 350 e.p.g. On 19-1-40 and 20-1-40 two successive doses of 9 grammes each were given in the form of the 1 gramme tablets in gelatine capsules. The day after the second of these doses the egg count was 0 and it remained so until 25-1-40. On this date and on 26-1-40, two further doses of 9 grammes of the same tablets were given and the egg count remained at 0 until 31-1-40, when it was 100. On 31-1-40 and 1-2-40 and 2-2-40, three successive doses of 9 grammes each of the same tablets were given. From 3-2-40 to 7-2-40 the egg count remained at 0, but was at 100 e.p.g. on 7-2-40. It remained at this level until 16-2-40 when the egg counts were discontinued until 8-4-40 when the count was 0 again, although this lamb had been in the same box with the Marked Lamb, which still had an egg count of 50 to 100 e.p.g.

The undosed ewe had a mean egg count on the first three days of the experiment of 1,350, 2,700 and 1,800 respectively, but by the fifth day it had fallen, without dosing, to 600 and thereafter fluctuated between 400 and 800 e.p.g. until 7-12-39, when it was killed off by mistake. It did not therefore provide a very satisfactory control. The fall in its egg count without dosing is, however, a point to be noted, because this frequently happens in all kinds of animals and must be considered when the egg count is used as a criterion of the effect of any anthelmintic.

The conclusion drawn from this experiment was that a dose of about 0.3 gramme per lb. body weight removed most of the worms of a light infestation, but that a few remained, which it was difficult to remove with phenothiazine, perhaps because the animals were confined in a box and probably self-and cross-infestation were occurring all the time. Animals in the field would be subject to much heavier infestations after dosing, so that it would be even more difficult, if not impossible, to reduce their egg counts to 0 and to maintain it so. The reduction of the egg counts to 0 for three or four days was not regarded as significant, because egg counts are notoriously fallible as evidence of the degree of an infestation. In any event, the failure to produce a negative egg count over long periods may carry with it the advantage that the retention of some worms by an animal helps to maintain its resistance to further infestation. On the other hand, Gordon (1939) (*Austral. Vet. J.* 15. 118-120) has pointed out that in a sheep harbouring, say 8,000 *Haemonchus*



*contortus*, half of which may be females each producing 5,000 eggs per 24 hours, a 90 per cent. reduction of the worm burden still leaves 800 worms, 400 of which can be calculated as being females, so that this sheep will still be voiding 2,000,000 eggs in 24 hours—a considerable daily infestation of the pasture if it is multiplied by a number of sheep in a flock.

## 2.—EXPERIMENTS ON FLOCKS IN THE FIELD

A. Examination of two lambs sent in to the Institute from a farm in Norfolk showed that one contained 3,600 *Ostertagia* in the abomasum and 6,400 *Nematodirus* in the intestine, and the other 35,000 *Ostertagia* in the abomasum and no worms in the small intestine. This flock was regarded as suitable for dosing and experiments.

The owner agreed that 50 animals (ewes and lambs) should be dosed with phenothiazine and 50 with copper sulphate (without nicotine) to compare the effects of these two anthelmintics, the remainder of the flock being dosed with copper sulphate without nicotine. The two lots of 50 ewes and lambs (Groups 1 and 2) were all marked by clipping the ears and each animal was weighed separately on a weighbridge before the dose was given. A faecal sample was taken from the rectum of each animal when the dose was given and these samples were mixed to provide composite samples for each of the two groups. Each of the lambs and ewes in Group 1 was given 5 grammes of phenothiazine in tablet form and each in Group 2 was given 2 oz. of a 1 per cent. solution of copper sulphate without nicotine. On the 10th, 20th and 30th days after the dose each lamb was again weighed on the same weighbridge and a faecal sample was taken from the rectum of each to provide a composite egg count for each of the two groups. The average weight of Group 1 was 53·8 lb. and that of Group 2 54·8 lb.

For the first 20 days the animals were folded on sainfoin and during the first ten days only they all received extra corn rations. For the final ten days they were moved from the sainfoin to tares, the sainfoin having become by then so much woodier in the hot, dry weather that the farmer considered this change advisable.

The following tables show the individual weights of the lambs, the total weights of each group and the egg counts on the composite faecal samples from each group.

TABLE I

*Group 1. Nos. 1-50. Weights stated in lb. Avoirdupois*

No.	Initial Weight 24-5-40 Before Dose of 5 Grammes Phenothiazine	Weight 3-6-40	Weight 13-6-40	Weight 24-6-40
1	57	68	71	79
2	48	56	64	67



No.	Initial Weight 24-5-40 Before Dose of 5 Grammes Phenothiazine	Weight 3-6-40	Weight 13-6-40	Weight 24-6-40
3	52	60	70	82
4	*53	57	62	71
5	64	70	65	85
6	65	74	73	86
7	63	74	80	86
8	31	34	38	46
9	*60	67	69	77
10	49	56	62	65
11	60	66	72	80
12	57	68	69	79
13	*53	64	71	81
14	68	76	82	90
15	48	56	60	65
16	67	76	83	88
17	*46	58	62	70
18	46	54	60	66
19	40	46	53	58
20	(22)	24	22	22
21	58	71	74	77
22	27	36	40	47
23	*48	58	64	71
24	52	60	66	71
25	66	76	84	87
26	*57	64	72	82
27	(32)	37	38	44
28	64	68	74	76
29	53	60	65	69
30	63	69	74	77
31	63	70	73	80
32	73	82	90	94
33	73	81	84	85
34	54	64	72	77
35	*57	68	75	69
36	58	63	72	82
37	63	76	82	87
38	55	60	66	71
39	45	53	58	61
40	59	66	72	76
41	48	56	60	64
42	60	70	74	82
43	55	64	70	74
44	45	58	66	73
45	51	58	64	71
46	55	62	64	70
47	47	57	56	70
48	55	68	73	79
49	50	60	66	70
50	58	68	64	73

*Group 2. Nos. 51-100. Weights stated in lb. Avoirdupois*

No.	Initial Weight Before Dose of 2 oz. of 1 per cent. Copper Sulphate	Weight 3-6-40	Weight 13-6-40	Weight 24-6-40
51	58	66	75	82
52	77	93	95	100
53	57	70	76	85
54	53	60	66	75
55	51	56	52	54
56	50	54	56	64
57	37	44	52	58
58	51	58	62	65
59	*53	63	68	76
60	42	50	55	65
61	63	64	70	77
62	70	84	90	91
63	53	58	60	70
64	22	28	30	36
65	50	54	60	64
66	66	72	79	84
67	76	85	92	101
68	50	56	66	73
69	13	16	18	20
70	*64	76	78	89
71	51	62	65	72
72	46	50	57	61
73	*45	55	63	70
74	53	64	76	82
75	49	58	63	65
76	50	57	62	66
77	66	78	85	90
78	57	64	73	78
79	53	60	61	64
80	58	66	64	70
81	54	63	66	76
82	63	70	80	87
83	30	34	38	40
84	60	70	70	74
85	60	71	78	89
86	*44	50	59	68
87	*50	58	66	72
88	*50	58	65	71
89	59	67	64	80
90	73	86	90	96
91	52	60	67	73
92	56	66	73	82
93	51	56	64	67
94	71	80	90	92
95	50	60	68	76
96	61	70	76	87
97	54	60	60	63
98	54	60	64	64
99	76	88	96	100
100	56	64	68	73

TABLE II

*Total weights in lb. Avoirdupois*

	Group 1. Nos. 1-50. Given 5 Grammes Phenothiazine.			Group 2. Nos. 51-100. Given 2 oz. of 1 per cent. Copper Sulphate.		
	Total Weight.	Increase.	Eggs per Gramme Average of 2 Counts.	Total Weight.	Increase.	Eggs per Gramme Average of 2 Counts.
24-5-40	2,693		1,050	2,708		2,150
3-6-40	3,107	414 Average 8.3	150	3,012	304 Average 6.08	400
13-6-40	3,440	333 Average 6.6	50	3,369	357 Average 7.1	369
24-6-40	3,642	302 Average 6.04	100	3,677	308 Average 6.1	250

It will be seen that the weight increases of individual lambs differed considerably, and that some of the animals at times lost a few pounds in the intervals of ten days. The main fact emerging from the experiment is that there was no significant difference in the progress of the weight increase in the two groups. The slight advantage on the 20th day in favour of the group given copper sulphate and its disappearance by the 30th day should be noted here, in view of the results of the next two experiments. It could be argued that the dose of phenothiazine that was given was small, a normal therapeutic dose being 10 to 30 grammes. Yet the egg count of Group 1 was reduced by this small dose in about the same proportion as the reduction of the higher egg count of Group 2 by copper sulphate. It could equally well be argued that, in spite of the higher egg count of Group 2, about twice as high as that of Group 1, the copper sulphate enabled this group to maintain a weight increase equivalent to that of Group 1 given a small dose of phenothiazine. The experiment does not tell us whether, if the dose of phenothiazine had been higher, Group 1 would have shown a markedly greater increase of weight than that attained by Group 2. Another experiment, now in progress, may help to answer this question. Meanwhile, this experiment shows that even 5 grammes of phenothiazine has a significant effect on a comparatively heavy infestation with *Haemonchus*, *Ostertagia* and *Nematodirus*.

It has been suggested [Singer and Baker. (1940.) *Cornell Vet.* **30**. 375-382 and others] that *Nematodirus* is one of the nematodes (*Trichuris* and *Ancylostoma deudendale* may be others) which are comparatively little affected by phenothiazine, but the writer has as yet seen no evidence in favour of this view.

B. Experiment A was repeated on a group of ewes and lambs belonging to this Institute, but the egg counts on the composite faecal samples taken before dosing were low, so that little information was expected from this experiment about the relative efficiencies of phenothiazine and copper sulphate. Further, accidental obliteration of some of the markings prevented the keeping of an accurate record of the weight of individual animals. The two groups A and B were kept separate, grazing on different fields about a mile apart.

The following table (*see next page*) gives the relevant data about these animals. It shows that the weight increase during the 23 days following the

dose was small. Some animals, like some of those in experiment A, lost a few pounds. Both copper sulphate and phenothiazine reduced the egg count of the ewes to 0, while the egg counts of the lambs were not appreciably affected by either anthelmintic.

It will be seen that in this experiment, unsatisfactory though it was, there was, 23 days after the dose, a slight advantage in weight increase in favour of the group given copper sulphate. In experiment A there was a similar advantage, 20 days after the dose, in favour of the group given copper sulphate. By the 30th day this had disappeared. In the experiment next to be described, the group given phenothiazine attained, by the 30th day, a total weight approaching twice that attained by the group given copper sulphate and nicotine.

C. One hundred lambs, three to four months old, were included in this experiment. On 5-7-40 one lamb of this flock had been sent to the Institute for *post-mortem* examination. A worm count done on it showed 2,100 *Ostertagia* in the abomasum and 50,000 or more *Nematodirus* in the small intestine. Egg counts on the contents of the colon and rectum showed 13,800 eggs of *Nematodirus* per gramme and 3,500 eggs per gramme of other nematodes, presumably *Ostertagia*.

TABLE III

	Total Weight Before Dose on 27-5-40.	Composite Egg Count Before Dose on 27-5-40.	Total Weight on 20-6-40 (23 days later).	Composite Egg Count on 20-6-40 (23 days later).	Total Gain.
	<i>Group A. Given Copper Sulphate (10 to 15 c.c. of 5 per cent. solution according to body weight)</i>				
Ewes	2,092 lb.	350 e.p.g.	2,186 lb.	0 e.p.g.	94 lb.
Lambs	875 "	50 "	1,033 "	150 "	Average 1.9 158 lb.
	<i>Group B. Given Phenothiazine Tablets (in the proportion of about 0.1 gramme per lb. body weight).</i>				
Ewes	2,033 lb.	850 e.p.g.	2,094 lb.	0 e.p.g.	61 lb.
Lambs	686 "	450 "	821 "	500 "	Average 1.6 135 lb.
					Average 2.7

On 26-6-40 the 100 lambs were divided into Groups 1 and 2 of 50 lambs each. Each lamb of Group 1 was given 5 grammes of phenothiazine in tablet form (cf. experiment A); each lamb of Group 2 was given 5 to 8 c.c. (according to the size of the lamb) of a solution containing 10 per cent. of copper sulphate and 10 per cent. of a solution containing 40 per cent. of nicotine sulphate. McEwen has informed the writer that he gave for his experiments reported elsewhere in this symposium, 20 c.c. of a solution containing 5 per cent. of copper sulphate and 5 per cent. of a solution containing 40 per cent. nicotine alkaloid. His dose of copper sulphate and nicotine was thus rather more than that given to the lambs used in this experiment. McEwen, however, gave much larger doses of phenothiazine in his experiments, giving 30 grammes to each lamb in his experiment 1 and 10 grammes to each in his experiments 2 and 3.

Faecal samples were taken with the finger from the rectum of each lamb before it was dosed.

The total weight of each group of lambs was ascertained by weighing the lambs on a weighbridge. It was discovered that the weighbridge recorded a



difference of 4 to 6 lb. in the weight of each animal according to whether it stood at one or other end of the platform of the machine, while an intermediate weight was recorded when it stood in the middle of the platform. This possible error was reduced to a possible error of 2 lb. or so per lamb by inducing each lamb to stand at the same end of the platform while its weight was being recorded. This experience showed that it is possible to introduce a considerable error into any experiment involving the use of a weighbridge, unless the position of the animal on the platform of the machine is always the same. Another possible source of error in the weights is the normal variation in the weight of each animal before or after a feed or access to water; they should always be weighed at the same time in the day. Further, when faecal samples are being taken from each lamb, it is inevitable that different amounts of faeces will be taken from each, either because the finger cannot take equal amounts from each, or because the second 50 lambs in a group of 100 will have been waiting longer and will often have emptied the rectum before the sample is taken or because the faeces of some are looser than those of others, so that the finger removes less and there are relatively fewer eggs in the amount of solid removed. When, therefore, all the samples are mixed to form a composite sample on which an egg count is made, it may occur that the egg count is really done mostly on the faeces of the first 50 of the group and mostly on the best-formed faeces of these. It may therefore not represent the average egg content of the faeces of individual lambs.

On the 10th, 20th and 30th days after the doses were given, the lambs were again weighed and faecal samples were taken. Throughout the experiment the lambs were grazing on grass with the rest of the flock and had no extra feed. The following table gives the relevant facts about them.

TABLE IV

	Group 1. Given 5 Grammes Phenothiazine in Tablet Form.			Group 2. Given 5 to 8 c.c. of a Solution Containing 10 per cent. of $\text{CuSO}_4$ and 10 per cent. of a Solution Containing 40 per cent. Nicotine Sulphate.		
	Total Weight.	Increase.	Eggs per Gramme Average of 2 Counts.	Total Weight.	Increase.	Eggs per Gramme Average per 2 Counts.
26-6-40	2,898 (before the dose)		650	3,162		400
5-7-40	3,367	469 Average 9.38	200	3,837	675 Average 13.5	550
15-5-40	3,200	Loss 167 Average 3.3	200	3,464	Loss 373 Average 7.46	400
26-5-40	4,208	Increase 1,008 Average 20.16	350	3,980	Increase 518 Average 10.3	400
		Total gain in 30 days 1,310. Average 26.2			Total gain in 30 days 818. Average 16.3	

No satisfactory explanation was found of the considerable loss of weight during the second ten days in both groups but especially in Group 2. It may



have been partly an apparent loss due to the fact that the lambs were weighed on the first two occasions in the morning and on the second two occasions in the afternoon and that in the afternoon they may have been emptier of grass and water and therefore weighed lighter than they would have done in the morning. But this could hardly have accounted for the whole of the loss of weight recorded, nor for the fact that the loss was twice as much in the group given copper and nicotine. The main fact emerging from this experiment is, nevertheless, that the total gain of weight in 30 days attained by the group given phenothiazine was substantially greater (approaching twice as much) than that of the group given copper sulphate and nicotine. This happened in spite of the fact that only a small dose of phenothiazine was given, namely 5 grammes, whereas 10 to 30 grammes is a normal therapeutic dose. This experiment showed, therefore, that, although at the end of the first ten days the group given copper and nicotine (like the groups given copper sulphate only in experiments A and B) had gained more weight than the group given phenothiazine, by the end of 30 days the group given phenothiazine had done considerably better than the group given copper and nicotine, and a good deal better than the corresponding group in experiment A. This conclusion is supported by a comparison of the composite egg counts. The small dose of phenothiazine reduced this and kept it down at about the same level for 30 days, while copper and nicotine (a normal therapeutic dose) did not appreciably affect it. It may be noted here that Singer and Baker (1940. *Cornell Vet.* **30**. 375-382) found that phenothiazine was more effective than copper sulphate and nicotine or tetrachlorethylene against nematode infestations of sheep.

Further experiments are desirable to ascertain the accuracy of the impression given by this experiment (and to a lesser degree by experiment A also) that the effect of phenothiazine lasts much longer than that of copper and nicotine. If this should prove to be true, the other experiments on sheep and horses, described in this paper, will have an additional interest because they show that egg counts reduced by phenothiazine do not necessarily rise again for many weeks, although the animals are out at grass and are presumably being continuously re-infested.

## GOATS

So far only one goat has been available. This was a goat weighing 62½ lb., belonging to a member of the staff of the Imperial Chemical Industries, Manchester. Samples of its faeces were sent by Dr. J. R. M. Innes, who subsequently dosed it with 10 grammes of phenothiazine without toxic effect. Before the dose was given the faeces showed an egg count of 3,650 eggs per gramme (average of two counts).

A sample of faeces collected 24 hours after the dose showed an egg count of 100 (average of two counts). Fourteen days and eighteen days after the dose the egg count was still 100 e.p.g. and 18 days after the dose it was 50 e.p.g. (average of 2 counts). Further samples of faeces will be examined to find out whether the egg count, like that of the lambs and ewes described above, will remain at this low figure for a considerable time.

*Attempts to Kill the Ciliates in the Rumen of Sheep with Phenothiazine*

On 22-4-40 counts were begun of the ciliates in the rumen of a sheep with a fistula into the rumen. The fistula had been made by Mr. Phillipson, of this Institute, some weeks previously and the sheep was in good bodily condition, weighing 258 lb.

The number of ciliates present was estimated only roughly because it was difficult to count organisms so active as these. The method was to withdraw 2 c.c. of rumen contents, mix it with 98 c.c. water and remove 0.1 c.c. with a pipette. This was diluted with an equal quantity of Lugol's iodine, which killed the ciliates. The ciliates were then counted with the aid of an eye-piece in which hairs crossed at right angles were fixed to divide the field into four parts. The number counted was multiplied by 500 to give the number of ciliates per c.c. After the dose of phenothiazine, the ciliates already dead were first counted in the drop. The iodine was then added to kill the rest, and the number of ciliates was counted again, so that the number alive and presumably not affected by phenothiazine could be estimated. Egg counts were also done, but not more than an average of 50 e.p.g. were found so that the anthelmintic effect of the phenothiazine was ignored.

The daily ciliate counts were :—

22-4-40	576,266	27-4-40	570,000
23-4-40	484,132	28-4-40	Not done
24-4-40	1,463,066	29-4-40	1,013,000
25-4-40	543,000	30-4-40	574,500
26-4-40	448,000		

On 30-4-40 four gelatine capsules, containing six tablets each containing 1 gramme of phenothiazine—a total dose of 24 grammes of phenothiazine—were placed in the rumen through the fistula opening. The sheep thus received a dose of about 0.09 gramme per lb. body weight. Six hours later it was passing red urine. The gelatine of a capsule put into rumen contents in a flask on the previous day and incubated at 38° C. was dissolved in half an hour, and the phenothiazine was set free. It was therefore reasonable to assume that five and a half hours after the phenothiazine became free in the rumen the animal was passing thionol in the urine.

Ciliates were counted in samples of rumen contents taken one, four and twelve hours respectively after the capsules were administered, but no appreciable decrease in the living ciliates was noted. No toxic effects whatsoever were observed in this sheep. The conclusion was therefore that a dose of 0.09 gramme of phenothiazine per lb. body weight directly introduced into the rumen through a fistula did not have any appreciable effect on the ciliates of the rumen.

## CALVES

Only one series of calves has been treated. All the calves were about six months old, but their egg counts were not high. They were all dosed at the farm, no change being made in the conditions under which they were kept there. They were all given the first phenothiazine dispersion issued by Imperial Chemical Industries. The table (overleaf) shows the results, and it will be

have been partly an apparent loss due to the fact that the lambs were weighed on the first two occasions in the morning and on the second two occasions in the afternoon and that in the afternoon they may have been emptier of grass and water and therefore weighed lighter than they would have done in the morning. But this could hardly have accounted for the whole of the loss of weight recorded, nor for the fact that the loss was twice as much in the group given copper and nicotine. The main fact emerging from this experiment is, nevertheless, that the total gain of weight in 30 days attained by the group given phenothiazine was substantially greater (approaching twice as much) than that of the group given copper sulphate and nicotine. This happened in spite of the fact that only a small dose of phenothiazine was given, namely 5 grammes, whereas 10 to 30 grammes is a normal therapeutic dose. This experiment showed, therefore, that, although at the end of the first ten days the group given copper and nicotine (like the groups given copper sulphate only in experiments A and B) had gained more weight than the group given phenothiazine, by the end of 30 days the group given phenothiazine had done considerably better than the group given copper and nicotine, and a good deal better than the corresponding group in experiment A. This conclusion is supported by a comparison of the composite egg counts. The small dose of phenothiazine reduced this and kept it down at about the same level for 30 days, while copper and nicotine (a normal therapeutic dose) did not appreciably affect it. It may be noted here that Singer and Baker (1940. *Cornell Vet.* 30. 375-382) found that phenothiazine was more effective than copper sulphate and nicotine or tetrachlorethylene against nematode infestations of sheep.

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seen that the egg counts of all those which were dosed except No. 6, were reduced by the dose to 0, but the egg counts of the controls were also very low, so that this result is inconclusive. The results obtained with the marked and unmarked lamb (recorded above) show that it is unlikely that the egg count reduced to 0 would have continued at 0 for very long and that it would probably have been very difficult to keep the egg count at 0. It is likely that 50 to 200 e.p.g. would have remained even after repeated doses. The chief value of this experiment was that it showed that no toxic effects were observed in calves six months old when doses of 80 to 125 grammes of phenothiazine were given.

TABLE V.

Date.	Kept in a barn.		Kept Outside in a Compound on Straw.					
	No. 1.	No. 4. Contr.	No. 5.	No. 6.	No. 7.	No. 8 Contr.	No. 9. Contr.	No. 10. Contr.
20-10-39	500	No faeces	300	200	100	400	200	100
21-10-39								
22-10-39								
23-10-39	600 Given 100 grammes Phenothiazine	100	0 Given 125 grammes Phenothiazine	150 Given 100 grammes Phenothiazine	100 Given 80 grammes Phenothiazine	200	200	500
24-10-39								
25-10-39								
26-10-39	100	200	100	No faeces	100	200	0	500
27-10-39								
28-10-39								
29-10-39								
30-10-39								
31-10-39	0	50	50	250	0	50	200	150
1-11-39								
2-11-39	0	100	0	100	0	100	0	400
6-11-39	0	0	0	50	0	100	50	400

## HORSES

PONIES DRENCHED WITH PHENOTHIAZINE.—Three Shetland ponies and one New Forest pony were offered for experiment by Dr. Hammond of the University of Cambridge School of Agriculture. All these animals were grazing and were kept in the field during the experiment. They ranged in age from three to five years, but the weight of only one was ascertainable at 664 lb. Two were kept as controls and two were given 80 grammes and 60 grammes respectively of the first phenothiazine dispersion issued by Imperial Chemical Industries. This corresponded to a dose of about 0.1 gramme per lb. body weight. No toxic effects were observed, but the day after the dose red urine was passed and the mucosae of the eyes, nose and mouth took on a bronze coloration which gradually waned until it had disappeared four to five days after the dose. It was no doubt due to thionol in the blood.



The egg counts were reduced immediately, dropping from the region of 1,000 to 1,500 until, three days after the dose, they were negative. They remained negative until the ninth day after the dose, when the egg counts were discontinued. After the dose many dead ciliates were found in the faeces. This suggests that phenothiazine kills ciliates which live, as those of the horse do, in the caecum and colon, although the introduction of phenothiazine directly into the rumen contents of a sheep had had no effect on the ciliates there (as recorded above). This is a detail which supports the view expressed by other workers that phenothiazine has more effect on parasites which live in the caecum and colon, a view which suggests that phenothiazine cannot act immediately, but requires time to undergo some change before its toxic action on nematodes is exerted.

#### HORSES DOSED WITH PHENOTHIAZINE POWDER IN BRAN AND TREACLE.—

(1) A longer series of observations was made on a New Forest pony weighing 714 lb., belonging to this Institute. This pony was out at grass throughout the observations and was given 60 grammes of phenothiazine in bran and treacle. The phenothiazine powder now available was not being made when this experiment was done, but 60 tablets, each containing 1 gramme of phenothiazine, were broken up and mixed with the bran and treacle. The egg count showed only strongyle eggs, and although the egg count was not very high, the mean of two daily counts ranging from 350 to 1,150, it had become 0 on the third day after the dose. Egg counts were continued every two or three days and, although the pony was out at grass, they remained negative until the 41st day after the dose, when 150 e.p.g. were present. On the 44th day after the dose 250 e.p.g. were found, and on the 48th day 100 e.p.g. The pony was therefore then acquiring a slight reinfestation. No toxic effects whatever were observed in this animal. It grazed on the same pastures until 24-6-40, when the average of two egg counts was 400. A further infestation was thus being established then.

(2) *Thoroughbred 3* was given 40 grammes of phenothiazine powder, prepared in a similar way, a dose of about 0.2 gramme per lb. body weight, in bran and treacle with a similar result. The mean of two daily egg counts on this horse ranged from 150 to 800 for a period of 23 days before the dose. This horse was kept indoors in a box throughout the observation. The day after the dose, its mucosae were bronzed, but were practically normal again in four days and quite normal seven days after the dose. The blood of this horse was examined spectroscopically on the first and second days after the dose, but nothing abnormal was found in it. No toxic effects were observed. The egg count became negative on the second day after the dose and remained so until the eleventh day after the dose when the experiment was discontinued.

#### THOROUGHBREDS GIVEN PHENOTHIAZINE POWDER IN WATER BY STOMACH TUBE TO TEST ITS TOXICITY

*Thoroughbred 2*.—This mare, two years old, 952 lb. in weight, was given 500 grammes of phenothiazine powder obtained by crushing up in water tablets each of which contained 1 gramme of phenothiazine. The suspension was given through a stomach tube. The object of this very large dose, amounting to

rather more than 0.5 gramme per lb. body weight, was to test the toxicity of the drug. There were so few eggs in the faeces that this animal was not suitable for observation of the anthelmintic efficiency of the drug. On the day after the dose the animal was off its food and was passing red urine. The mucosae were bronzed and there were petechial haemorrhages in the vulva around the vestibule, possibly due to irritation by the urine. On the second day after the dose, the animal was normal again and remained so until the fourth day after the dose, when the mucosae were practically normal in colour. The animal had evidently suffered no serious ill-effects from this large dose.

Forty-nine days after this large dose, a second dose of 500 grammes was given, also by stomach tube. This time the phenothiazine powder that had just been introduced by Imperial Chemical Industries was used in water. This large dose was given in the morning. At 3 p.m. the same day the animal was uneasy, showed evidence of intestinal discomfort, and would not feed. The mucosae of its eyes and mouth were already bronzed and the urine thick and chocolate-coloured. Possibly the intestinal discomfort was due to the mechanical effects of the bulk of the drug in the stomach, because by the next morning the animal was feeding well and seemed fairly fit. The mucosae were still bronzed and the urine red. Blood submitted to Professor Keilin for spectroscopic examination showed an increase of about 10 to 12 per cent. of methaemoglobin which suggested that the phenothiazine had produced some oxidation of the haemoglobin, but the amount of methaemoglobin was not accurately estimated. No sulphaemoglobin was present and the blood did not show any other abnormality. On the following day the methaemoglobin was only slightly reduced, but by 16.1-40, eight days after the dose, it had practically all disappeared. In blood, taken two days after the dose, the haemoglobin index was 83 per cent. and the red cell count 6,037,500, the colour index being 0.7; two days later the haemoglobin index was 78 per cent. and the red cell count 6,818,000, the colour index being 0.58. On the eighth day after the dose, the haemoglobin index was 81 per cent., and the red cell count 7,000,000 and the colour index 0.578. These figures are within the normal variations shown by the blood of healthy horses.

The day after the dose, a number of dead strongyles were recovered from the faeces, some containing eggs, others being flattened and pigmented brown. These were identified as mature and immature *Trichonema* spp.

On the second day after the dose, the animal was still listless, but brighter than the day before. Its temperature was 92.2°, and the limbs were cold. It ate hay, but little else; the mucosae were still bronzed and the urine red; in the afternoon the temperature had risen to 100.2° and a bran mash was refused. On the third day after the dose the animal was still listless, with a morning temperature of 100° and an afternoon one of 99.2° and red urine and bronzed mucosae. Rather more food was taken and the condition was on the whole better. On the fourth day after the dose the temperature was as for the third day, the mucosae almost normal, the urine still red and the animal was eating hay only.

On the fifth day after the dose the morning temperature was 100.6°, the mucosae about the same, the urine still red, but the animal was much brighter and was feeding better.

On the seventh day after the dose the mucosae were still slightly bronzed, especially above the teeth, the urine was still red and the bowels loose, but the animal was feeding well and in quite good condition.

By the eighth day after the dose, when the spectroscopic blood examination showed that very little methaemoglobin was left, the urine was still red. It did not become normal till the tenth day when the mucosae were also normal and the animal had fully recovered.

This history is interesting because it shows that a second large dose of 0.5 gramme per lb. body weight given to a young horse was definitely toxic, whereas the previous dose had had little toxic effect. The interpretation of this result is difficult, but it is worth noting, because it may indicate that successive doses must be given with care. It is possible that one dose may sensitise an animal for some time. On the other hand, the dose given to this horse was excessively large and its very bulk may have caused most of the trouble. There is no record of ill-effects in horses from repetition of the small doses and there is evidence in the literature that, in other animals, repeated small doses do not have toxic effects and may be more effective than larger single doses. The experiments with pigs recorded below suggest that the age of animals dosed is an important factor. A note on the effects of relatively large doses recently given to young foals by F. Day, F.R.C.V.S., will be sent to the *Veterinary Record* as soon as possible.

*Thoroughbred 1.*—Another mare, 9 years old, and weighing 1,232 lb., was given 1,000 grammes of phenothiazine powder obtained by crushing up in water tablets each of which contained 1 gramme of phenothiazine. The suspension was given by stomach tube, the object of the very large dose being to test the toxicity of the drug. There were so few eggs in the faeces that this animal was not suitable for observation of the anthelmintic efficiency of the drug. The day after the dose was given, this animal would not feed and was passing red urine and its mucosae were bronzed. It was restless and in some pain all day. On the second day after the dose it seemed better and one dead *Ascaris* was found in the faeces. Early on the third day after the dose, the animal was very restless and still would not feed. At 2-30 P.M. she was down and struggling for breath with staring eyes and was obviously in great distress. She was got up, but walked very stiffly. The temperature was 103°, the pulse rapid and weak, she was sweating a little and the ears were cold. At 4-30 P. M. she was destroyed.

The following extract from the note published by Innes and Whittick on this horse shows that, in their opinion, most of the symptoms shown by this animal were due to a thrombo-angiitis, rather than to the phenothiazine. It is difficult, however, to resist the conclusion that the condition found in the stomach and kidneys was caused by the drug. The case must remain inconclusive, but it is clear that a dose as high as 1 gramme per lb. body weight should not be given to horses. It is not likely ever to be given, because the experiments done with other horses show that 0.1 gramme per lb. body weight is an efficient anthelmintic dose.

Extracts from—INNES, J. R. M., and WHITTICK, J. W. (1940.) *J. Path. and Bact.* 50, 377-381. "Thrombo-angiitis obliterans in a Horse."

Summary of *post-mortem* findings :—

“ Pulmonary embolism ; thrombosis of termination of the abdominal aorta ; occlusion of the large arteries of both hind limbs by thrombus and mural thickening ; thrombosis and thickening of some veins in the hind limbs ; numerous acute haemorrhagic erosions in fundus of stomach ; subendocardial haemorrhage in left ventricle and right atrium ; congestion of lungs ; areas of mucosal necrosis and ulceration in renal pelves and ureters, probably attributable to the toxic action of the drug ; no oedema of the limbs.”

On subsequent page the authors say :—

“ There was no evidence of verminous infestation in any of the vessels.

“ The vascular lesions are definitely those of organized thrombosis and, in the absence of cardiac disease and of arteritis due to *Strongylus* larvae, the assumption of primary arterial disease of the vessels of the hind limbs seems to be justified.

“ Thrombosis of the veins in this horse was probably secondary to slowing of the blood flow consequent upon the arterial obstruction.

“ The arterial changes, because of their chronicity, cannot be attributed to the action of phenothiazine, although the drug in the dose given may have been toxic and may have precipitated the final illness.”

## PIGS

In the colons of two pigs received at the Institute, from a farm, nodules resembling those produced by *Oesophagostomum* were found, although no worms or larvae could be found in them.

Phenothiazine powder, derived from the breaking up of 1 gramme tablets, was given to these pigs. They were all about three months old and their average weight was 35 lb.

The pigs were divided into Groups A, B and C of ten pigs each and a group of undosed controls. The pigs in Group A were dosed and weighed individually, each pig being given 0.5 gramme per lb. body weight of phenothiazine. For the pigs in Groups B and C enough phenothiazine was weighed out to give the pigs in Group B 0.2 gramme per lb. body weight and those in Group C 0.1 gramme per lb. body weight. The total dose for each group was then mixed in the trough with the food and the pigs were allowed to eat it, the intention being to repeat the dose of 0.1 and 0.2 grammes per lb. body weight if no ill effects were seen. The pigs took the doses well, but there was no certainty that each pig got an equal amount of phenothiazine.

*Group A, given 0.5 Gramme per lb. Body Weight, Dosed Individually.*—The day after the dose one pig of this group was brought to the Institute unconscious and waving its legs in the air. It subsequently recovered by the fourth day. Other pigs were similarly affected, but less seriously, showing only drunken gait, inability to turn corners and weakness of the hind legs. All of these subsequently recovered. The dosing was not repeated.

*Group B, given 0.2 Gramme per lb. Body Weight as a Mass Dose in the Feed.*—The day after the dose one pig only showed some slight weakness in the hind legs. A second dose of 0.2 gramme per lb. body weight was given on the



second day. On the third day six pigs showed complete loss of co-ordination, but recovered by the fourth day. The dose was not repeated after this second dose.

*Group C given 0.1 Gramme per lb. Body Weight as a Mass Dose in Feed.*—After the first dose no pigs showed any symptoms and on the second day a second dose of 0.1 gramme per lb. body weight was given. The day after this second dose three or four pigs showed slight rolling gait and inco-ordination, but all recovered. The dosing was not repeated, so that these pigs had had two doses on successive days of 0.1 gramme per lb. body weight, 0.2 gramme per lb. in all.

Two months later it was adjudged that all the pigs which had had phenothiazine were in better condition than those which had not. The weight increase, however, was roughly the same in the dosed groups and in the undosed controls.

The impression gained from this inconclusive experiment was that the method of dosing was responsible for the ill-effects observed. Only a few of the pigs were adversely affected, which suggested that these obtained more food and therefore more than their allotted dose of phenothiazine. This the writer had expected, but it was thought worth while to see if this method of dosing was safe in practice. The following additional experiment suggests that an overdose of the drug obtained in this way was probably the correct explanation of the ill-effects.

Twenty-seven pigs about three months old, undergoing a metabolism experiment at this Institute, with an average weight of 42 lb., were each given individually 0.1 gramme per lb. body weight of the 5 gramme tablets of phenothiazine now being made by Imperial Chemical Industries. The pigs were starved for 24 hours before the dose was given. They were being fed individually so that they could be dosed individually by mixing the dose with the feed.

None of the pigs showed any visible ill-effects. None showed any inco-ordination. The exact weighings of each of the ingredients of the rations that were being given to them indicated that the food intake of some of the pigs was reduced after the drug was given, although no visible effects on the animals could be noted. After five days all the pigs had resumed their normal intake of food. There is as yet no clear proof that this reduction in food intake was due to the phenothiazine.

Two other pigs of the same lot as the above 27 and the same age were also dosed individually as follows :—

One weighing 61 lb. was given 0.1 gramme per lb. body weight of phenothiazine tablets and showed no visible ill-effects. Four days later, when its urine was no longer red, it was given 0.5 gramme per lb. body weight without visible ill-effects.

The other, weighing 37 lb. was given 0.14 gramme per lb. body weight without visible ill-effects and four days later it was given 0.2 gramme per lb. body weight without visible ill-effect.

These observations, inconclusive as they are, do indicate that pigs three months old dosed individually with 0.1 gramme phenothiazine per lb. body weight do not suffer any visible ill-effect. When the dose for a number of pigs



is mixed in the feed and the pigs are allowed to take it as they will, there is danger that some will get too much and will suffer accordingly.

Larger doses will in the near future be given to pigs individually and the results will be compared with those of Swanson, Harwood and Connelly (1940) (*J. Amer. vet. med. Ass.* **96**, 333-338), who gave up to 1 gramme per lb. body weight to older pigs without toxic effects. Meanwhile, it is probably wise to give phenothiazine to *young pigs* with great care. The *Ascaris* burden of the pigs described above was not removed, nor much influenced, by this dose of phenothiazine. The reason for this is not clear.

#### SUMMARY

(1) Two lambs weighing 74 lb. and 89 lb. were given 0.3 gramme and 0.27 gramme per lb. body weight respectively of phenothiazine in the form of tablets. Three days after the dose the egg count had fallen, in the one case, from 7,400 e.p.g. to 200 e.p.g. and, in the other, from 3,850 to 550 e.p.g., these egg counts being the mean of two counts done by different observers on different portions of the same sample of faeces. The egg counts of both lambs, which were kept in a box together, remained round about this figure for rather longer than five months. Attempts to reduce the egg count to 0 and to maintain it at 0 by giving further doses of phenothiazine were not successful.

It is never desirable to remove all the nematodes from any animal because this procedure will lower or remove the host's resistance to the infestation.

(2) Comparisons of the effect of phenothiazine, copper sulphate and copper sulphate and nicotine on nematode infestations of ewes and lambs in the field indicated that small doses (about 0.1 gramme per lb. body weight) of phenothiazine lowered the egg counts more effectively than did either of the other two anthelmintics and tended to maintain them more effectively at a lower level. In three experiments 157 ewes and lambs were weighed every ten days for a period of 30 days after doses of about 0.1 gramme per lb. body weight of phenothiazine. The results indicated that, even when these small doses are given, the weight increase of animals given phenothiazine is significantly greater at the end of 30 days than that of animals given normal therapeutic doses of copper sulphate or copper sulphate and nicotine, although, during the first 20 days or so, the animals given these two latter anthelmintics appear to put on slightly more weight. The egg counts of the animals given phenothiazine were reduced more effectively than those given the other two anthelmintics and remained low. It is thus possible that phenothiazine has a more lasting anthelmintic effect than copper sulphate and copper sulphate and nicotine, perhaps because it prevents the development of a second generation of worms picked up from the pasture. Further experiments on this question are in progress.

It is unlikely that a copper deficiency in the animals used contributed in any way to these results, because there was no evidence of this and swayback had never been reported from the district in which the animals used were bred.

(3) No toxic effects were shown by any of the sheep treated with these doses of phenothiazine. It was not expected that the small doses used would have any toxic effects.

(4) A similar marked fall in the egg count of a single goat from 3,650 e.p.g. to 150 e.p.g. was noted 24 hours after it had been given, without toxic effect, a dose of 0.15 gramme per lb. body weight of phenothiazine.

(5) Attempts to kill the ciliates in the rumen of a sheep by introducing phenothiazine powder into the rumen through a fistula did not kill any appreciable number of the ciliates. Thionol appeared in the urine of the sheep 5½ hours after phenothiazine was set free in the rumen.

(6) Doses of 80 to 125 grammes of phenothiazine did not produce any visible toxic effect in calves six months old.

(7) The passage of red urine and bronzing of the mucosae, which usually persists for four days or so after the administration of phenothiazine, was noted in all the sheep, horses and calves treated. When the doses were larger, loss of appetite and listlessness were noted. These are signs that the dose should not be increased, nor repeated until a considerable interval has elapsed.

(8) Phenothiazine powder at the rate of about 0.1 gramme per lb. body weight administered to horses, either mixed in a feed of bran and treacle or given suspended in water with a stomach pump, reduced the strongyle egg count to 0, or near that figure, and the egg count did not rise again appreciably for many weeks, although the horses were at grass.

(9) One horse, given 500 grammes of phenothiazine in water with a stomach pump (a dose of about 0.5 gramme per lb. body weight) suffered little more than transient loss of appetite and listlessness. After the same dose 49 days later the same horse became restless and uncomfortable and refused to feed for a few days, but subsequently recovered. Another horse, given 1,000 grammes with a stomach pump showed marked toxic effects, but the existence in this animal of thrombo-angiitis rendered it impossible to say whether the symptoms were due to this disease or to the phenothiazine. Examination of the blood of these horses showed no other abnormality than a transient excess of methaemoglobin.

(10) Evidence is given which suggests that phenothiazine should be administered to young pigs with great care, although doses of 0.5 gramme per lb. body weight appear to have no toxic effects on mature pigs.

# HIGH RECORDS CONTRASTED WITH UNSELECTED RECORDS AND WITH AVERAGE RECORDS AS A BASIS FOR SELECTING COWS\*

BY

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WHILE making some preliminary studies of suitable ways to test the relative values of production records, singly and in various combinations, for predicting future records of the same cow and for evaluating her transmitting ability, we noted an article by Copeland (2) purporting to show some advantages for using a cow's highest record as an index to her lifetime producing ability and to her transmitting ability. As our data, which came from a different source, seemed appropriate for testing Copeland's conclusions, we repeated a part of his analysis on our own material and added a few other features which, it is hoped, will demonstrate why the *ex post facto* selection of the highest record is an unsound procedure—a procedure more likely to lead to fallacious results than if an unselected record or, better still, the average of all a cow's records were used as the measure of her ability.

A major difficulty associated with the use of the highest record in comparing cows (and not mentioned by Copeland) is that in practice most comparisons will need to be made between cows which have not completed an equal number of recorded lactations at the time the comparisons must be made and which, therefore, have not had the same number of chances to make a high record. The obvious unfairness so introduced seems of so much practical importance that we will refer to it again in some detail after first presenting our findings on a basis comparable with Copeland's.

## DATA STUDIED

We studied the records of 115 Holstein cows, each of which had completed at least six Herd Improvement Registry lactations and had one or more daughters each of which had completed at least two records<sup>1</sup>. All records were brought to a mature 'B' basis by use of the conversion factors listed in the Holstein Red Books.

## FINDINGS

The first step was to compare the correlations between various records and averages. These are shown in Table 1 along with the corresponding ones

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<sup>1</sup>These data were those included in some originally assembled for another purpose—that of proving sires. Here a 6-record cow was used as many times as she had tested daughters. Actually there were 89 different cows. If the dam of a 6-record cow had two or more records, that dam was treated as a daughter. The relation is a parent-offspring one in either case. The findings should be practically the same as if there had actually been 115 different 6-record cows, each with one tested daughter.

reported by Copeland. For the four 'repeatability' comparisons (the first four in Table 1), our coefficients are lower than those reported by most students of this question, while those of Copeland are among the highest we have ever seen. More typical are findings like those of Plum (5) that a correlation of .60 existed between records of the same cow in the whole population of records from many herds, but that on an intra-herd basis the correlation between records of the same cow was reduced to .40. Perhaps a high degree of heterogeneity from herd to herd existed in Copeland's data.

TABLE I

*Correlation coefficients for Holstein H. I. R. records Jersey R. of M. records and Jersey Herd Test records*

Variates correlated	Coefficients of correlation		
	115 Holstein H.I.R. cows	197 Jersey R. of M. cows*	166 Jersey Herd Test cows*
First record with the second	.30	.71	.78
Second record with the third	.42	.77	.80
Third record with the fourth	.55	.69	.75
Fourth record with the fifth	.45	.59	.83
First with ave. of next four	.49	.62	.80
First with ave. of all five	.68	.75	.88
Highest with ave. of all five	.90	.92	.92
Highest with ave. of other four	.83	..	..
Lowest with ave. of all five	.79	..	..
Lowest with ave. of other four	.70	..	..

\*Copeland's data.

If the various records were all equally correlated ( $r$ ) with each other, the correlation between the first and the average of the next four would be expected to be  $r \sqrt{\frac{4}{1+3r}}$ . It is a little lower than this (if for  $r$  is used the simple arithmetic average of the four repeatability correlations shown) in all three sets of data (especially so in the Jersey R. of M. records), thus indicating that the correlations between non-consecutive records (not shown) average somewhat lower than those between consecutive records. Similarly, if all the correlations between the individual records were equal to  $r$ , the correlation between the first record and the average of the first five records would equal  $\sqrt{\frac{4r}{5}}$ . Again the actual figures are lower (much lower in the case of the Jersey R. of M. records) than they would be if the non-consecutive correlations averaged as high as the consecutive ones which are shown.



The numerical values to be expected in the case of the correlation between the highest record and the average of all five are not clear because of the complex statistical relations brought about by the *ex post facto* selection of the highest record. That such selection has an effect on the correlation subsequently calculated can be made clear by considering an extreme case in which it is assumed that we have samples of five records each from cows between which there really are no intrinsic differences, the variation between the various records being wholly due to varying external circumstances and to sampling variations. This is the same statistical problem as would exist in

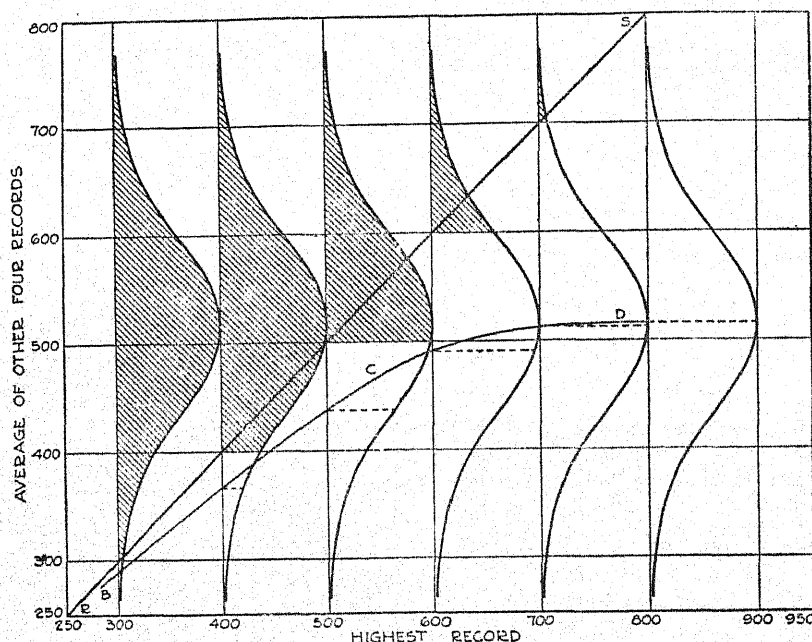


FIG. 1. The statistical consequences of the *ex post facto* selection of the highest record among five. The normal curves, all drawn at the same height on the ordinate axis, illustrate the variation to be expected between different records of the same cow in a population of cows which all have exactly the same real ability. In such a population it would sometimes happen that the highest of five records would be very high; sometimes it would be moderately high; and sometimes even the highest record would be moderately low. When a sample of five records is located along the abscissa according to the highest record in it, the other four must come from the unshaded portions of the frequency curves—i.e. from below the line RS. When the highest record is large, it can be considerably larger than the average of the other four records; but when the highest record is itself rather low, it cannot be very different from the average of the other four. This gives rise to a high correlation between the high and the average of the others, to curvilinear regression, and to a funnel-shaped distribution.

studying random samples of five records each, drawn from the same universe. Since all of the other four records must be lower than the high record, it follows that the samples in which the high record itself has a low value will be samples in which all five records happened to come from the lower part of the frequency curve. But in such cases the other four will rarely be *much* below the high one and the two variates will be closely correlated.



Figure 1 is intended to show graphically what would happen in such a case if the highest of the five records in each sample were correlated with the average of the other four. Six normal curves with their means at the same level have been drawn at intervals of 100 lb. along the abscissa to show the restrictive effect of locating the sample along the abscissa according to the highest of the five records in it. In each case the other four records must all be below the diagonal RS, and must come from the unshaded portions of the normal curves. At the right hand side of the graph (where the high record happens to be very high) little restriction is laid on the magnitude of the other four records, but toward the left hand side most of the unshaded portion of the curve lies very near the diagonal RS and the average of the other four records cannot be far below the high record.

Because of the constricting influence of the high record on the magnitude of the other four, the total distribution becomes funnel shaped and the line BCD (drawn through the means of the unshaded portions of the six normal curves) showing the regression of the average of the other four on the high one is curvilinear in character. The extreme narrowing of the distribution when the high record itself is not very large, is particularly potent in giving a large correlation between the high record in the sample and the average of

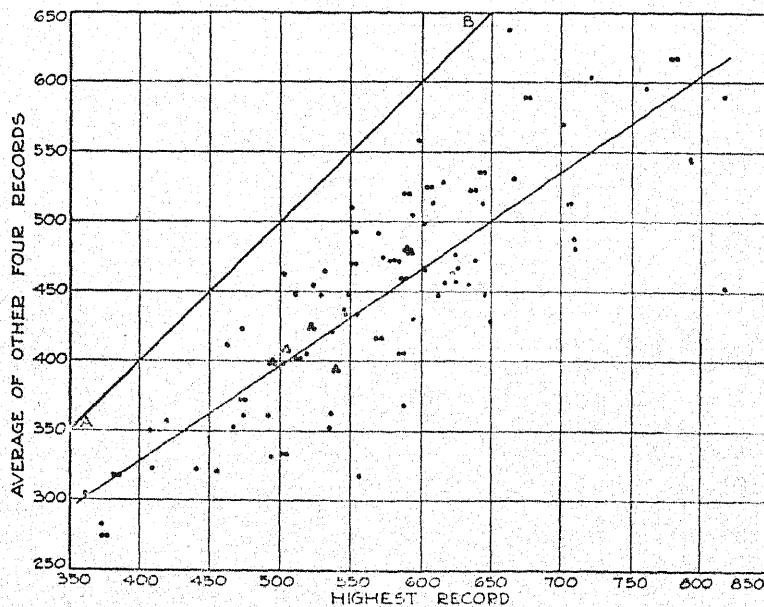


FIG. 2. Correlation (.83) between the highest record and the average of the other four records in a population of 115 H. I. R. cows. All points must fall below the diagonal AB. The regression line is the best-fitting straight line but does not tell the full story, since the real relation between the two variates is curvilinear. The restrictive influence of the highest record on the magnitude of the other four records is especially evident towards the left side of the diagram.

the other four records, even in an extreme case like this where the samples come from the same universe (as from a group of cows all with the same producing ability).

Actually, of course, there are differences between the cows, too, and this gives the regression line more of an upward slope and somewhat less curvilinearity although the funnel shape of the data and the extreme restriction at the left are conspicuous features of the actual data as shown in Figure 2. It is thus apparent that the correlation coefficient for the high record and the average of the other four owes a part of its magnitude merely to the fact that the first mentioned variate was selected *because* it was the high record. Because of the curvilinearity and the funnel shape, the biometric relations seem complex and we do not know how to separate the observed correlation coefficient into the spurious part which comes from this useless *ex post facto* arrangement of the data and the part which would show the genuine usefulness of the high record in predicting records not yet made when the high one was selected.

When the highest record is correlated with an average of the five which include it, the coefficient becomes still larger. The situation is the same as shown in Figure 2 except that each point is now moved upward one-fifth of the distance which in Figure 2 separates it from the line AB. Naturally those lying farthest away are moved most and so the correlation is distinctly increased but no new knowledge is gained.

Figure 3 shows the first record as correlated with the average of the other

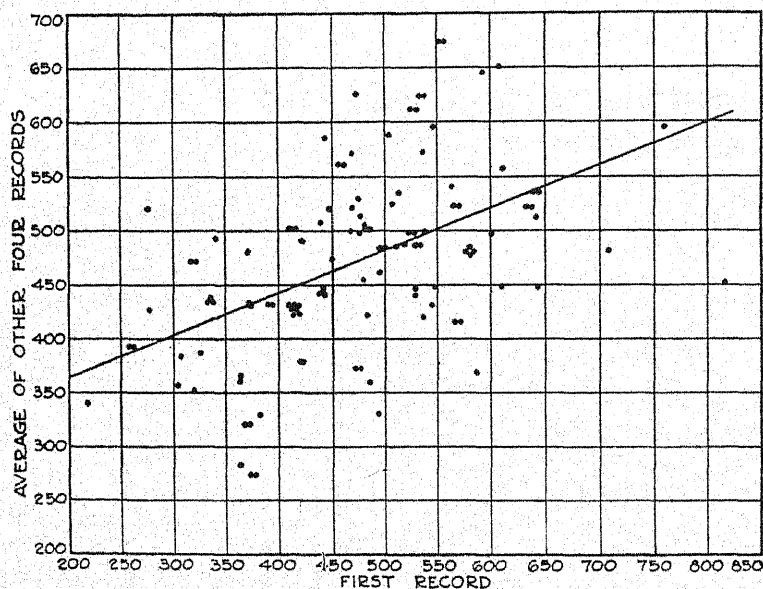


FIG. 3. Correlation (.49) between the first record and the average of the next four records. Same cows as in Figure 2. The straight line shows the linear regression of the average of the other four records on the first record.

four. Using the first record sets no restriction on the magnitude of the average of the other four. The other four can all be below the first, or all above it, or some on one side and some on the other. Such a correlation in an unbiased estimate of the usefulness of the first record for predicting the average of the other four.

## WHY NOT THE LOW?

To demonstrate more clearly how subsequent selection of a record *because* of its size affects the correlation coefficient, the lowest of the five records was correlated with the average of the remaining four and also with the average of all five. In our data the low record was less variable ( $\sigma = 82$ ) than the high ( $\sigma = 96$ ) and hence had less influence in determining the variation of the averages of five. For that reason alone, the low might be expected to be somewhat less closely correlated with the average than was the highest record. Moreover, the forces which tend to make the highest record large and those which tend to make the lowest record small may be different enough that the corresponding covariances may not be entirely comparable. For example, some of the low records may have been made under circumstances definitely known to have been abnormal but not specific enough that it would be practical for us (or for the breed association) to undertake any corrections for these circumstances. (Although this is plausible, it seems not to have been important, because the low records averaged only 102 pounds below the average of all five, whereas the high records averaged 98 pounds above. Therefore the distribution seems to have been nearly symmetrical). While the coefficients for the lowest record ( $.79$  and  $.70$  in Table I) are not as large as those for the highest record, yet they are much larger than correlations involving unselected records. The reasons are the same in principle as those discussed in connexion with Figures 1 and 2.

Before passing from this consideration of the lowest record some comment might be ventured relative to the practical considerations involved. Dairymen frequently raise objections to including all records of production in averages because they feel that some lactations are abnormal. Such objections are usually raised, however, *after* the cow has completed (or nearly completed) a low record. That is, it is difficult (in practice usually impossible) to know whether the circumstances were judged abnormal solely because of them or largely because it was seen that the record was going to be low. Theoretically, the omission of records known to have been made under definitely abnormal conditions for which adequate corrections cannot be made would, we think, enhance the value of the resultant averages. However, practical difficulties connected with deciding what constitutes grounds for omission, and with making sure that the size of the record itself does not influence the decision to use it or omit it, render the advisability of omitting any records from lifetime averages problematical. Certainly the reasons for such omission would have to be so specific and important that the decision in each case would be automatic. For example, in Denmark only those records made in years when the cow aborted or when she had foot-and-mouth disease are omitted from her average.

## PREDICTION OF FUTURE RECORDS OF THE SAME COW

The utility of the high record, as compared with that of unselected records may be tested in the following way freed (we think) of those statistical fallacies which beset of the correlation of the high record with the others *from which* it was selected. The highest, lowest and medium records among the

first three were compared with the fourth and with the fifth; and then the unselected first, second and third were correlated also with the fourth and fifth records. The results of such a fair test on our data are shown in Table II. The differences between the correlations in the same column are not statistically significant (on this amount of data—probably the question should

TABLE II

*Correlation coefficients between a cow's first three records (selected and unselected) and her later records*

The six variates correlated with the fourth and fifth records	Correlation coefficients with	
	Fourth record	Fifth record
Highest of first three . . . . .	.61	.29
Lowest of first three . . . . .	.56	.45
Medium of first three . . . . .	.57	.31
First record . . . . .	.39	.39
Second record . . . . .	.56	.24
Third record. . . . .	.55	.26

be investigated on more extensive data), but the contrast with the figures in Table I shows clearly that much of the apparent superiority of the high record (and of the low) in that table was due to the spurious effects of the method of its choice.

#### PREDICTION OF DAUGHTER'S RECORDS

Table III shows the correlations of the average of the first two records of the daughters with the high and low of all five records, with the high, low and medium of the first three, and with the five unselected single records. For comparison, Copeland's coefficients are included in this table. All the coefficients are below .34. The considerable differences between some of them do not seem to fit any particular pattern and are not statistically significant on this amount of data. So far as these data go, any one of these records is about as useful as any other for predicting the production of daughters.

#### USE OF AVERAGES

Averages of all available records are theoretically more useful than single records in evaluating the differences between cows. The authors are at present studying, on a larger amount of data, the question of whether records actually do behave in this way. Some of the preliminary results are presented in Tables IV and V.



TABLE III

*Correlation coefficients between the average of the first two daughter records and various records of the dam*

Dam's record which was correlated with the average of the daughter's first two records	Correlation coefficients
Highest of all five records . . . . .	.29
Lowest of all five records . . . . .	.15
Highest of first three records . . . . .	.24
Lowest of first three records . . . . .	.19
Medium of first three records . . . . .	.33
First record . . . . .	.24
Second record . . . . .	.14
Third record . . . . .	.23
Fourth record . . . . .	.29
Fifth record . . . . .	.13
Copeland's comparisons	
Highest records of 176 dams and highest records of the daughters	.29
Average records of 176 dams and average yield of their tested daughters . . . . .	.30

TABLE IV

*Correlations of averages of a cow's first records with her subsequent records and with her daughter's records*

Dam's average	Correlations coefficients					
	Dam's fourth record		Dam's fifth record		Daughter's ave. of first two	
	Actual	Exp.	Actual	Exp.	Actual	Exp.
Ave. of first two records . . . . .	.59	.47	.38	.47	.24	.25
Ave. of first three records . . . . .	.65	.51	.38	.51	.27	.27
Ave. of first four records . . . . .	..	..	.44	.53	.30	.29—
Ave. of all five records . . . . .	..	..	..	..	.29	.29+



TABLE V

*Correlation coefficients between the average of the last three records of six-record cows and the first three records taken singly and in various combinations*

Records correlated with the average of the last three records	Correlation coefficients	
	Actual	Expected
First record . . . . .	.42	.49
Second record . . . . .	.45	
Third record . . . . .	.46	
Average of first and second . . . . .	.54	.59
Average of first and third . . . . .	.53	
Average of second and third . . . . .	.54	
Average of first, second and third . . . . .	.58	.64

On comparing these tables with Tables I to III, it seems that there is a trend toward increased prediction value of average records as compared with single records.

The 'expected' values included in Tables IV and V are the ones which would have occurred if the various records of the same cow had all been equally correlated with each other and if they all had had equal standard deviations and if they had all been equally correlated with the daughter average. The correlation coefficients used in calculating the expected values in Table IV were the arithmetic means of the ten intercorrelations between the first five records (.39) and of the five coefficients between the cow's five records and her daughter's average (.21). In Table V, the inclusion of the sixth record gave a mean repeatability of .37.

The divergence of observed from expected values presumably was caused by actual inequalities among those various correlations and standard deviations and by the limited amount of data studied. The expected values, however, serve to indicate the magnitude of the increase which, theoretically, should occur as the correlation coefficients are based on averages of larger and larger numbers. Probably the results will become more regular when more data are included. The present amount of data is not large enough to be expected to show significant differences.

TABLE VI

*Distribution of the highest record of five over the five lactation classes*

Lactations	First record	Second record	Third record	Fourth record	Fifth record
Number of high records . . . . .	27	24	20	23	21

## CASE OF COWS WITH UNEQUAL NUMBERS OF RECORDS

Table VI shows that the highest record made by a cow is about as likely to occur in any one of the first five lactations as in any other. Assuming a perfectly even distribution of the high record, it follows that the probability that a cow with just one record has already made what would be the highest in five if and when she completed the additional four is .20; in the case of a cow with two records the probability is .40; for the three-record cow it is .60; for the four-record cow, .80; and for the cow with five records, the probability is, of course, 1.0. This shows clearly the unfairness of using the highest record as a basis for comparing cows which have completed unequal numbers of lactations.

The bias introduced by unequal numbers is evident also upon considering the ratio  $\text{range}/\sigma$ . The standard deviation ( $\sigma$ ) of records of the same cow, as calculated from our data, is 81.6 pounds. Using Tippett's (6) values for  $\text{range}/\sigma$  for varying values of  $n$  (the number of records) it is determined that with a cow like the average of ours when  $n = 2$  the high record is expected to be 46.0 pounds of butter fat above the mean (473.0 pounds) of the whole sample of 575 records. When  $n = 3$  the high record is expected to be 69.0 pounds above the mean; when  $n = 4$ , 84.0 pounds above; and when  $n = 5$ , 95.0 pounds above. In other words, the expected value for the highest record of two is  $473 + 46 = 519$  pounds; of three, 542; of four, 557; and of five, 568 pounds. The actual values for the average of the highest of the first three and of all five were calculated for the 115 cows of this study. They proved to be 545 and 571 pounds respectively—values that agree well with those expected theoretically. The bias of comparing the highest records only, when cows have different numbers of records, is obvious.

Copeland thinks it impractical to compare lifetime records of daughters and dams in proving bulls because the daughters and many of the dams will not have completed their lifetime of production at the time when, by using single records or by using only the highest records, it becomes possible to 'prove' the bull. In using lifetime records for proving bulls, one merely uses all the information available at the moment when a decision has to be made. In using all of a cow's completed records as her average it is no more necessary to wait until her lifetime is ended than it is necessary, when proving a bull, to wait until records have been made by all of the daughters he will ever sire. The greater the number of animals and the larger the number of records completed by them, the less the error likely to be involved in the 'proof'. That the figures for the proof next year or the year afterward may be a little different usually means only that the later figures, because of the additional records included, are a bit more dependable. Breeders of dairy cattle are continually faced with the necessity of making decisions concerning cows which have completed different numbers of lactations. The most dependable procedure is to use all the lactation records available at the time the decision needs to be made.

In comparing cows which have completed unequal numbers of records and which have been selected for comparison because their averages are high (as in choosing a son of a high producer), or because their averages are low (as when deciding which of two cows to cull), it is necessary to take account of

the decreased variability of averages based on large numbers. Otherwise it would almost always appear that the best producers—and also the worst producers—were cows with only one or two records. The fairest way of doing that seems to be to estimate each cow's real productive ability as equal to the herd average plus (or minus)  $\frac{nr}{1+(n-1)r}$  times the excess (or deficit) of her average over the herd average.\* In this formula  $n$  is the number of lactations included in her average and  $r$  is the repeatability of records of the same cow—a figure which is usually around  $+.3$  to  $+.4$  on an intra-herd basis.

When it is  $.4$  the above fraction reduces to the simple form  $\frac{2n}{3+2n}$ . In its use a cow with one record is credited with  $2/5$  of her apparent superiority, one with two records is credited with  $4/7$  of the apparent superiority of her average, etc. (Similarly in case her average is below the herd average, her real productivity would be estimated at  $2/5$ ,  $4/7$ , etc., as far below the herd average as her own actual average is.) This makes it possible to compare, without bias from differences in the number of records they have completed, cows which were selected because their records were high or because they were low. No such precaution is needed in proving sires because their daughters must be unselected (if the proof is to have much validity) and the dams will not often have been selected intensively enough for such a discounting to be important. Usually there will be both low and high producers among the dams (and among the daughters) and the errors from not discounting those with high records will tend to cancel the opposite errors from not discounting those with low records.

#### SUMMARY

The high correlation between a cow's highest record (or her lowest) and the average of the other records from which this one was selected results largely from the statistical effects of this selection itself. This high correlation does not indicate superiority of the selected record for predicting future records or breeding value. When the highest record is correlated with other records from which it was *not* selected the resulting coefficient (provided all cows have the same number of records) indicates that the high record is of somewhere near the same reliability as an unselected record but almost certainly less reliable than the average of all unselected records. Differences in number of completed records, however, is of so much practical importance in making selected records unfair that the use of the highest record, as an indication of a cow's lifetime producing ability, cannot be recommended.

Averages appear to be more dependable than either selected or unselected single records for evaluating differences between cows.

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\*For three independent derivations of this formula, each from a slightly different point of view, see references (1), (3) and (4).

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## ABSTRACTS

### Prevention of ovine mastitis by the use of staphylococcus toxoid. F. C. MINETT (1939). *J. Comp. Path.* 52, 167

RECENT knowledge on the rôle of antitoxin in staphylococcal immunity had not been exploited to ascertain if the course of infection in staphylococcus mastitis in animals can be modified through prior intervention with toxoid. The author has, therefore, now tried to supply experimental data on the point. The sheep was employed as the experimental animal for testing the extent to which specific toxoid treatment might modify the course of acute staphylococcus mastitis and in order to gain experience of the kind of toxoid that should be used and the antitoxin response likely to be aroused.

It is essential to note that the sheep for use in these experiments were selected after a preliminary titration of their blood sera for natural staphylococcal  $\alpha$ -antitoxin. Only those with low titres of antitoxin were selected for experiment. In some experiments, however, sheep with higher antitoxin level were used for toxoid treatment, as it was anticipated that the toxoid treatment might act as a secondary stimulus in such sheep and so lead to a higher state of immunity.

Two types of toxoid were used for immunisation trials, a formalised toxoid and an alum-precipitated toxoid. The toxoids were used subcutaneously and where two doses were given, an interval of about 3 weeks was allowed. After an interval of about 10 days after injection of the first or second dose of toxoid, the treated animals along with an adequate number of controls were tested for immunity by instilling into one or both sides of the udder a mixture of toxin and living culture of a virulent staphylococcus. In one experiment the interval was prolonged to about 3 months to see if immunity could be demonstrated after that interval.

Samples of serum were collected from the experimental animals before coming under experiment and again at intervals after treatment with toxoid and after administration of the test dose, with a view to follow how the level of anti-toxin in these animals fluctuated and how these fluctuations were correlated with the state of the resulting immunity. In the article the results as regards these fluctuations are summarised in tabular form and again illustrated graphically; also the degrees of resulting mastitis are illustrated diagrammatically.

In all, five experiments were conducted and they were designed to test the relative efficacy of the following methods of immunisation: (1) single large dose of formal toxoid; (2) two large doses of formal toxoid; (3) single small dose of alum-precipitated toxoid; (4) two small doses of alum-precipitated toxoid; (5) a single large dose of alum-precipitated toxoid; and (6) two large doses of alum-precipitated toxoid.

The following observations were made:—

- (1) Following the injection of the test dose, control sheep took very little food during the first three or four days. The day after injection they suffered from some degree of lameness which persisted for a few days in some of them. The udders were swollen and tense and many sheep suffered from necrosis of the tissue and skin of the udder. A few even died or had to be destroyed on humane grounds. But in animals submitted to treatment with toxoid the general effects were reduced to a considerable extent. Unprotected sheep with low antitoxin titre suffered more severely from the test inoculation than those with higher titres.
- (2) In some experiments there was a relationship between the size of the local reaction following the test inoculation and the subsequently developed serum titre. It was in the direct ratio in control sheep and in the inverse ratio in the immunised sheep. Thus in the control group the local reaction was largest in animals which later showed a high antitoxin response. In the toxoid group the higher antitoxin response was associated with smaller local reactions.

- (3) Under the conditions of the experiment two injections of toxoid at an interval of about three weeks gave a better immunity than a single dose. Immunity was evident for at least three months after the second injection. In a single experiment one large dose of alum toxoid gave a rather better immunity response than two doses of unprecipitated toxoid and the smaller amounts of alum toxoid.

The amount of antitoxin developing in sheep treated with toxoid tended to be greater in animals with higher pre-injection titres. In sheep judged to be adequately protected antitoxin levels averaging 8 to 24 units per c.c. serum were observed 10 to 20 days after treatment, representing increases of 6 to 15 times the pre-injection titre.

[V. R. R.]

**An insectarium with constant temperature and humidity control, together with a description of a simplified technique for the rearing of *Anopheles maculipennis* var. *atroparvus*. D. S. BERTRAM AND R. M. GORDON (1939). *Ann. Trop. Med. Parasit.* 33, 279**

IN the first part of the paper the authors describe the construction and equipment of an insectarium recently built at the Liverpool School of Tropical Medicine, in which moisture is supplied by two humidifiers regulated by a humidity control apparatus, and heat by means of electric heaters provided with thermostats. The room has a capacity of about 1,000 cubic feet, and did not prove very expensive to construct. The solar heat that enters through a window is a source of difficulty in this contrivance. It was found impossible to maintain a constant temperature without the use of blinds, since sudden rises in temperature of as much as 5°C. (9°F.) occurred on sunny days even in early summer. It was possible to maintain constantly a relative humidity between 75 and 85 per cent so long as reasonable precautions were taken to see that the door of the insectarium was not left open unnecessarily. No arrangement is made for reducing humidity. The room is used for maintaining stocks of various biting insects.

The second part of the paper describes a method for rearing *Anopheles maculipennis* var. *atroparvus* in the insectary. The authors have followed Bate's method in principle. This consists in making a thick suspension of soil and adding it to the breeding bowl at frequent intervals. This method is simple and very successful.

[B. C. B.]

**The anterior pituitary lobe hormones in the treatment of ketosis in the dairy cow. M. G. FINCHER AND C. E. HAYDEN (1940). *Cornell Vet.* 30, 197**

THE pituitary gland is closely related to the function of the reproductive glands and the udder. There is a definite relationship between high milk production, poor reproduction and acetonemia.

It has been suggested that one of the functions of the anterior pituitary lobe hormone (A. P. L.) is to control or regulate the carbohydrate metabolism. Ketosis is believed to be due to disturbance in carbohydrate-fat metabolism. The primary seat of ketone body production is the liver and the A. P. L. acts upon the liver and prevents the formation of ketone bodies.

The writers studied the effect of A. P. L. upon the acetone body content of the blood and urine in definite cases of acetonemia. The experiment was conducted on seventeen clinical cases of acetonemia. The quantitative test for acetone bodies in their blood and urine indicated that these animals showed varying degrees of acetonemia.

The anterior pituitary lobe hormone was administered to thirteen of these cases. Two animals were administered gonadotropes in the form of gonadin. One of the two remaining animals received anterior pituitary-like preparation and the other animal was administered the anterior pituitary lobe preparation in addition to the anterior pituitary-like preparation. Additional treatment such as glucose, molasses and chloral was given to some of these cases.

Some cases responded very well to the treatment and it became evident from the chemical examination of blood and urine of treated cases that A. P. L. had some definite merit and it appeared to be the only treatment required in some cases. The writers admit that the data obtained by them are too few to substantiate the claim that these hormone preparations are specific in the treatment of ketosis though in some cases their use appeared to have had some merit especially as a few of their serious cases of ketosis recovered with no other therapy.

In most severe cases of acetoneuria in valuable cows where the speedy return to high milk production is desirable it would appear wise to combine the use of A. P. L. with dextrose, calcium gluconate and chloral. These writers believe that the anterior pituitary lobe hormones may have a definite place in the treatment of disorders that afflict the dairy cow at or near parturition. [P. R. K. I.]

**Maintenance protein requirements of sheep.** (a) **The endogenous nitrogen metabolism of sheep with special reference to the maintenance requirement of protein.** D. B. SMUTS AND J. S. C. MARAIS (1938). *Onderstepoort J. Vet. Sci. & Anim. Indust.* **11**, 131. (b) **The endogenous nitrogen metabolism of young sheep with special reference to the estimation of the maintenance requirement of sheep.** D. B. SMUTS AND J. S. C. MARAIS (1939). *Onderstepoort J. Vet. Sci. & Anim. Indust.* **13**, 219.

(a)

THE authors subscribe to the view that the maintenance requirement of nitrogen of an animal can be measured by the total nitrogen excretion in the urine after the endogenous level is attained. On this basis the endogenous urinary nitrogen excretion of mature Merino wethers was determined. The nitrogen-free diet consisted of dextrinised starch, agar, cod-liver oil, bone ash and salt. A constant level of endogenous nitrogen excretion, viz. 0.041 gm. per kg. body-weight was reached in from 6-15 days of nitrogen free feeding, the length of this period depending on the protein content of the diet on which the animals were kept prior to nitrogen free feeding. The distribution of the urinary end products, e.g. creatinine, total sulphur and neutral sulphur at endogenous level was also determined. On the result of this experiment the maintenance requirement of a 100 lb. sheep for digestible protein was calculated to be 23 gm.; this figure is rather less than that advocated in the usual standards.

(b)

In this study an experiment similar to that in (a) was conducted on young Merino wethers with a view to establishing their maintenance requirement. The animals were put for three weeks on a 14 per cent protein ration previous to nitrogen free feeding. In this experiment it was found necessary to include some wheat straw and to reduce the agar in order to ensure an adequate energy intake. The endogenous level was reached on the 5th or 6th day of experimental feeding, in contrast with 14 days with mature sheep. The endogenous nitrogen excretion was found to be 0.051 gm. per kg. for four months old wethers. This corresponds to a requirement of 29 gm. digestible protein for a 100 lb. sheep. A formula for determining the maintenance requirement of sheep was suggested by the authors and its application tested. Moreover, it was shown that the basal metabolism of sheep can be predicted from the endogenous nitrogen, and that the figures arrived at agree very well with the values published in the literature. [R. M.]

**Dry skim milk in rations for growing, laying and breeding fowls.** W. H. OTT., H. C. KNANDEL AND R. V. BOUCHER (1939). *Pennsylv. Agri. Expt. Sta. Bull.* **381**, 1

ALTHOUGH the value of skim milk in poultry rations is widely recognised, there is comparatively little information regarding the optimum amounts which should be fed to various classes of poultry. This bulletin gives the results of an investigation carried out to test whether or not the addition of dry skim milk improves an already high grade and well balanced basal ration, and to ascertain the optimum amounts necessary.

Three experiments of 72 weeks duration each were carried out on Single Comb White Leghorns; the progeny of the first year's experimental birds were studied in the second year, the various groups being fed the same rations as in the first year. A fresh stock of birds was used in the final year. The birds in each experiment were equally distributed among eight groups and fed all mash rations in which the percentage of skim milk ranged from 0 to 8.75. The other constituents in the feed were suitably altered, within narrow limits, to maintain an average content of 16.9 per cent protein, 1.7 per cent calcium and 1 per cent phosphorus during the first 12 weeks of growth, and 14.4 per cent protein, 2.4 per cent calcium and 1.7 per cent phosphorus thereafter. Males were separated at 4 and 6 weeks of age and used for studying the shrinkage of live weight in shipping broilers.

The chicks were weighed at bi-weekly intervals till 24 weeks of age, and the mean weight in pounds per bird for each group has been presented. According to the authors, the beneficial effects of skim milk feeding were most pronounced in the first two weeks, and persisted till 10 weeks, during which period the gain in body-weight increased in proportion to the amount of milk fed; the differences in all cases, however, were relatively small. The efficiency of food utilisation appeared to be slightly better in the higher milk level groups in the early phase of growth, but this was counteracted in later weeks. When both efficiency of food utilisation and early rapid growth are considered, the best response was obtained from the groups fed 1.25 and 2.5 per cent milk.

Although the figures cited show no advantage in feeding skim milk beyond 10 weeks from the standpoint of body-weight alone, it is doubtful if its discontinuance would be beneficial in the long run. No data are included on the effects of such a procedure on subsequent egg production. The fact that among the laying hens in the second generation, age-at-first-egg decreased, and weight-at-first-egg increased with increasing levels of milk, and that mortality was significantly higher in the groups receiving 5 per cent and less of skim milk indicates the favourable effect of a liberal supply of milk.

Egg production of the birds in the first generation (1st and 3rd years of experiment) was more or less similar in all the groups, but in the second generation (2nd year of experiment) production was rather poor in the no milk and 1.25 per cent milk groups. There was no corresponding increase beyond the 5 per cent level in any of the years. Highest egg weights were obtained from the 3.75 and 5 per cent groups. Eggs from the 2.5 per cent and 3.75 per cent groups hatched as well as those laid by birds receiving higher amounts of milk. Taken together, these observations indicate that the optimum supplement of dry skim milk for laying hens is between 2.5 and 5 per cent, when the ration is otherwise well balanced.

The internal quality of the eggs was also tested in the first year on a random selection of 10 eggs from each group from one day's production. The factors studied were percentage of egg shell of total egg weight, percentage of ash in egg shell, percentage of yolk and firm albumin of total egg weight, albumin and yolk scores, and yolk index; but there were no significant differences in any of these factors in any of the groups.

In two experiments on the shrinkage of live weight in shipping broilers, the greatest shrinkage occurred in the no milk group, while the most satisfactory results were obtained in the 2.5 and 3.75 per cent milk groups.

[Y. V. N.]



## ANIMAL QUARANTINE NOTIFICATION

United States of America

THE following animal quarantine order has been received in the office of the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

*Order to prevent the introduction into the United States of rinderpest and foot-and-mouth disease dated 26th October 1940 issued by the Bureau of Animal Industry, United States Department of Agriculture.*

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The Editorial Committee of the Imperial Council of Agricultural Research, India, takes no responsibility for the opinions expressed in this Journal

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OUTKRAAL IN THE FOREST AREA AT MUKTESWAR

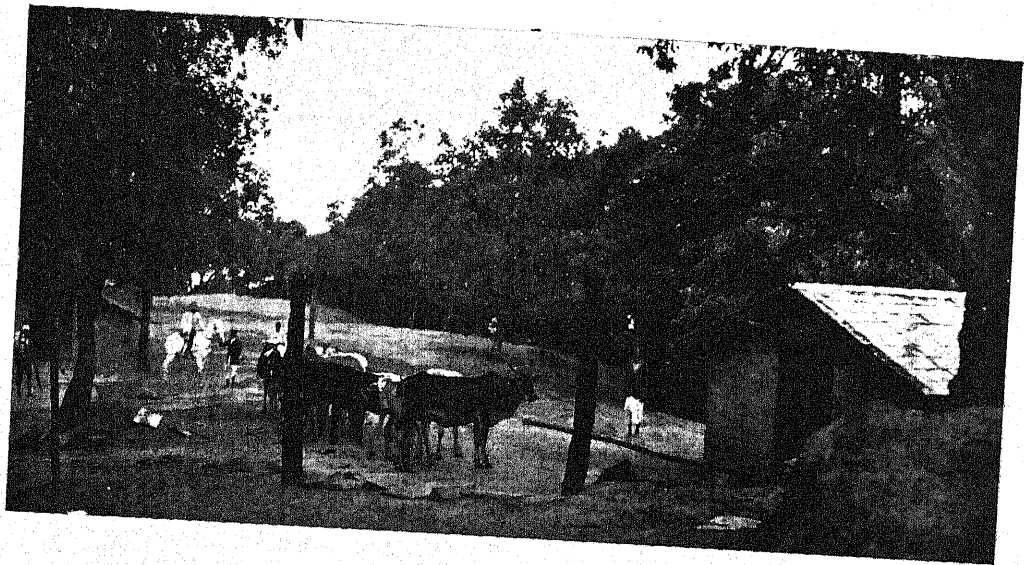


FIG. 1.



FIG. 2.

## ORIGINAL ARTICLES

### JOHNE'S DISEASE : TEN YEARS' OBSERVATIONS ON AN EXPERIMENTAL HERD

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(With Plate XX)

THIS herd was constituted in 1929 mainly with the following objects : (a) To observe whether under the conditions existing at Mukteswar the disease would spread from infected to healthy cattle, (b) to study the effect of treating a proportion of the animals with a live vaccine according to the method of Vallée and Rinjard [1926].

#### GENERAL DESCRIPTION OF THE EXPERIMENT

The herd was started in a shed in the main Institute area but after about a year the survivors were transferred to a particularly secluded out-kraal in the forest area of the Mukteswar Institute where the remainder of the experiment was carried on. This kraal (Plate XX) situated at an altitude of 6,125 feet consists of an area of land just less than  $\frac{1}{4}$  acre in extent, gently sloping from south to north, and is bounded by a wire fence. The soil is sandy and except in certain spots there is little tendency for rain to collect even during the monsoons. Seasonal variations in temperature are : November to March, min. 23°-37°F., max. 57°-66°F. ; April to July, min. 36°-52°F., max. 72°-80°F. The average annual rainfall is about 50 inches of which 20 to 25 inches fall during the summer monsoon from July to September. In April-June the daily average sunshine is about 12 hours, while in winter the average snowfall calculated as rain is 7 inches. The main shed measuring 40 by 12 feet usually housed 30 to 35 cattle of various ages and there was also a smaller shed. Both were ill-ventilated, with rough paved floors, and were used for housing the animals at night. During the day the animals were allowed to roam at will in the kraal, as well as in the adjoining forest for purposes of grazing. In fine weather they remained in the forest from 8 A.M. till 3 P.M. The calves were kept with their mothers and were not weaned, and the cows were not milked. On return from grazing the animals were given a diet of

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hay or grass and a little concentrate mixture (crushed gram, wheat bran, oil cake and salt). The roughage was fed on the floor of the sheds. Water was supplied from an iron tank filled from a stream running down the hillside and during the early part of the experiment drainage water from the sheds was also allowed to run into the tank. The cleaning of the sheds and of the outside enclosure was purposely not thorough and the floor of the sheds became slushy at the time of the rains and snow. All calvings took place in the same sheds, along with other animals. It was thought that these conditions in the sheds would give the infection every chance of spreading to the healthy animals, but it will be observed that the animals in other respects led a very natural life, were given a reasonably adequate maintenance ration and were not subjected to any physical strain, except at the time of calving. From time to time, the animals were tested by the double intradermal method with ordinary tuberculin and with avian tuberculin. Both tuberculins were prepared from glycerol broth cultures and were used in the heat-concentrated form. Finally, in April 1940, a test was made with ordinary tuberculin and Johnin, in both cases ammonium sulphate precipitated products from synthetic media and prepared in the Medical Research Council Laboratories, Mill Hill, England. From time to time also rectal smears and bowel washings were examined in a further attempt to locate infected animals. All animals which died or were destroyed were examined for Johne's disease by making microscopical preparations from the bowel wall, particularly from the terminal portion of the ileum and from the ileo-caecal valve. At the conclusion of the experiment selected animals were slaughtered and attempts made to cultivate Johne's bacilli on a liver extract-*phlei*-egg medium:

#### COMPOSITION OF THE HERD

The herd has been gradually built up of (a) naturally infected cattle from outside, and (b) healthy cattle, and its numbers have been augmented by the progeny of these two groups of animals. Breeds represented were: Tharparkar, Hissar, Bihar, Assamese, Sindhi and cross-bred. The size of the herd increased from 3 in August 1929 to 110 in April 1940, when the experiment was closed. [Although the total number of animals passing through the herd has been stated as 110, only 95 are mentioned in Appendices 1 and 2, the remainder having either died or been killed as calves by wild animals.]

In August 1929 there were purchased from Bihar three naturally infected cows (Nos. 1, 2 and 3), all three being typical cases of Johne's disease in which acid-fast organisms could be easily demonstrated in rectal smears and faeces. The animals reacted to avian but not to ordinary tuberculin. Two of these cows (Nos. 2 and 3) died a few months later in spite of treatment with formalin and Johne's bacilli were isolated from both of them. The third cow (No. 1) attained good condition and ceased to react to avian tuberculin, although rectal smears were teeming with acid-fast organisms resembling Johne's bacilli. She died in May 1931 of Johne's disease.

A second batch of 9 cows (Nos. 4-10, 61 and 62), 1 calf (No. 11) and 2 bullocks (Nos. 12 and 13) were received from Bihar in 1930. All these animals, as well as cows 1, 2 and 3, were accommodated temporarily in a shed in the

main Institute area and were transferred during 1930 to the out-kraal. All of the second batch of animals, except the calf (No. 11) which was not tested, had been declared infected as a result of an avian tuberculin test at the farm of origin. On arrival at Mukteswar they were retested with avian tuberculin and all except two (cow 5 and calf 11) reacted. Bowel washings from all these animals, except calf 11, showed acid-fast bacilli which were regarded as those of Johne's disease. They were subsequently tested at intervals with avian tuberculin with varying results (Appendix 2). The two bullocks were proved at *post mortem* examination in June 1931 to have been infected with tuberculosis and Johne bacilli could not be isolated. Of the remaining animals, 7 cows and the calf are dead and the remaining 2 cows (Nos. 61 and 62) were still alive and in fair condition at the close of the experiment. At *post mortem* examination Johne's disease was confirmed in 4 of the 7 cows (Nos. 4, 7, 8 and 10) and in the calf which was then  $2\frac{1}{2}$  years old. Of the other 3 cows, one (No. 5) was a tubercular subject while in two of them (Nos. 6 and 9) neither tuberculosis nor Johne's disease could be confirmed.

In July 1930 a cow (No. 14) which had reacted strongly to avian tuberculin and weakly to ordinary tuberculin was received from the Central Provinces and on *post mortem* examination Johne's disease could not be verified.

In July and September 1937 there were received from Assam 18 animals (4 cows Nos. 15, 16, 63, 64; 14 bulls and bullocks Nos. 17—26 and 65—68). Four of these (cows Nos. 15 and 63 and bulls Nos. 18 and 20), had been declared positive reactors to avian tuberculin and the remainder were regarded as infected with Johne's disease. Of these, 14 are dead or destroyed (2 cows and 12 bulls or bullocks); 3 of them (bulls Nos. 17, 19 and 22) were proved to be infected with Johne's disease, but the disease was not confirmed in the remaining 11 animals at *post mortem* examination.

At intervals between September 1930 and 1938, 23 animals (16 cows and 7 bulls) from the Institute Dairy were added, as they were suspected of Johne's disease. Of these, 21 are dead or have been destroyed with the result that 13 (11 cows and 2 bulls) were confirmed as cases of Johne's disease and 8 (3 cows and 5 bulls) were apparently healthy. The remaining 2 cows are alive and in good condition.

Finally, there have been born into the herd 53 calves. Of these animals 30 died or were destroyed between August 1930 and May 1940. Twelve of them (Nos. 11, 46, 47, 48, 49, 53, 54, 55, 56, 57, 94 and 95) were proved to be infected with Johne's disease when they came to *post mortem* examination at ages varying from 1 to 7 years, while the remaining 18 were apparently healthy. The other 23 (5 males and 18 females) are alive and in good condition.

#### EFFECT OF CALVING

In some of the clinical cases the effect of calving was considerable. For example, 5 cows (Nos. 2, 3, 28, 29 and 32) died 1-5 months after calving. Though cow No. 6 died two months after calving in a very poor condition she was negative for Johne's disease at *post mortem*.

## VACCINATION

From 1930 onwards every other calf born into the herd was injected subcutaneously in the neck with living Johne bacilli from culture [Vallée and Rinjard, 1926] the injection being made within 24 hours of birth. In most cases the vaccine was prepared from a stock culture of the organism grown on a *phlei*-agar medium. Until 1933 each animal vaccinated was given 25.0 mg. culture suspended in 2.5 c.c. saline. From 1934 to 1940 the same amount of culture was mixed with 600.0 mg. sterile sand suspended in 2.5 to 5 c.c. liquid paraffin or vaseline oil. As expected, this produced an extensive firm and lasting swelling whereas there was no enduring local reaction to the saline suspension of bacilli. The fate of vaccinated animals and of the unvaccinated controls is summarized in Appendix 3. It will be seen that of 8 calves vaccinated with the saline suspension 3 developed Johne's disease while of 8 unvaccinated animals 4 became infected. Of 14 calves vaccinated with live bacilli mixed with the unabsorbable recipient one developed Johne's disease, while 2 out of 13 controls became similarly infected.

## RESULTS OF TESTS

Out of 60 animals in Appendix 2 (a), 46 reacted strongly or weakly to avian tuberculin at least once during the period of observation and of these 46 reactors, 24 were confirmed as cases of Johne's disease. Out of 14 animals which did not react to avian tuberculin, 9 were positive for Johne's disease. Ten out of 60 reacted to ordinary tuberculin but only 4 of the 10 had microscopic lesions of tuberculosis.

## DISCUSSION

At the time this experiment was started more than ten years ago no knowledge was available as to the ease or difficulty with which the disease might be disseminated. There was, however, a feeling that in view of what is known to happen on infected farms, spread might occur rather easily. Actually, as described above, the converse was the case, the disease dying out although apparently given every chance to spread. On reflection, however, and in the light of present knowledge of the subject of epidemiology and the influence of environment on the spread of disease, it is clear that the events in an experimental herd such as this were bound to be complex and difficult to interpret. In what follows, some attempt is made to analyse the position.

It may be noted in the first place that the herd was made up of animals received from different places, at different times and in various stages of infectivity. In the various groups of animals received and examined at *post mortem* the proportion found to be infected was as follows: Bihar 8 out of 13, Assam 3 out of 14, Mukteswar Dairy 13 out of 21, while out of 30 calves born into the herd 12 acquired infection. Two animals of Bihar, 4 of Assam, 2 of the Mukteswar Dairy and 23 born into the herd are alive and in good condition. Thus during 10 years 1930 to 1939, 36 known infected animals were added to the herd, viz. in each year 4, 3, 4, 4, 1, 2, 7, 5, 1 and 5. Four of these were destroyed and 32, including the 12 calves, died. In spite of this,

judging from the final tuberculin and Johnin tests carried out in April 1940 the disease had failed to extend. In April 1940, 33 animals, other than very young calves, were available for test, viz. 2 cows from Bihar, 6 animals from Assam, 4 cows from Mukteswar Dairy and 21 animals born in the herd of which 12 had been vaccinated. The only ones to react were the vaccinated animals. Following this test 6 animals (Nos. 67—70, 76 and 88), taken at random from the non-reacting group, were slaughtered and a careful examination of the intestine by microscopical and cultural methods failed to show any evidence of Johne's disease.

In seeking to explain the facts, two questions for consideration are the susceptibility of breeds of Indian cattle and the possibility of infected animals recovering under certain conditions. There is no record of experiments having been carried out on the susceptibility of Indian cattle to Johne's disease, but the disease has been reported from a number of provinces and it may be presumed that as with other cattle susceptibility is greatest in early life. M'Fadyean and Sheather [1916] for instance found that many individuals of the bovine species offer a marked resistance to infection with Johne's disease, that calves under 6 months take the infection more readily and that by 6 months of age calves are already becoming more resistant.

With regard to the second question, it is still uncertain whether complete recovery—in the sense of destruction within the body of all Johne's bacilli—occurs, but it is well known that apparent recovery, i.e. recovery in the clinical sense, occurs quite commonly when the environment (housing, feeding, climatic factors) is favourable. Thus, months or even years may elapse before symptoms appear in Johnin-reactors, while in infected European cattle after the age of 5 years there develops an immunity which in some animals is strong enough to prevent any marked decline in condition for a number of years.

Hagan and Zeissig [1933] record the remarkable instance of a cow, experimentally infected by feeding, which showed severe symptoms of Johne's disease and was in fact judged to be at the point of death but which improved and gradually reached normal condition, at the same time becoming a non-reactor to avian tuberculin, though still continuing to react to the complement fixation test. At autopsy, however, it was found that infection had not been eliminated since lesions and the characteristic bacilli were present in the ileum and ileo-caecal valve. It is perhaps not unreasonable to think that this animal might have recovered completely had she been allowed to live long enough. And if an occasional advanced case of the disease may recover, it is not unlikely that animals exposed to natural infection may often contract the disease and recover without reaching the advanced stage in which symptoms are produced.

That apparent recovery may take place in Indian cattle is suggested by Cooper and Srinivasan [1931] who observed that a number of cattle sent from Bihar to Mukteswar as suspected cases of Johne's disease owing to poor condition, diarrhoea and reaction to avian tuberculin, improved greatly when subjected to good housing and liberal feeding. Within 2 months all diarrhoea had stopped and it was then very difficult to realize that they were still infected with Johne's disease. Whether these were examples of true recovery



cannot be stated, and in the present herd no proof of real or complete recovery has been obtained, but the issue is often confused by the difficulty which is experienced, firstly in making a diagnosis at all in this disease and secondly, after making a positive diagnosis, in obtaining confirmation at *post mortem* examination. In this connection it is of interest to note that although several of the animals shown in Appendix I (Nos. 6, 9, 12, 13, 14, 38 and 68) are recorded as having shown acid-fast organisms in faecal or rectal smears, these were not recovered later at *post mortem*, either by microscopical or cultural examination. Serial No. 44 in the same appendix was artificially infected intravenously with a dose of pure culture of Johne bacilli and gave 3 positive reactions to the avian tuberculin test, yet on *post mortem* examination acid-fast organisms were not recovered.

Another point to be considered is the comparative value of avian tuberculin and of Johnin, applied by the double intradermal method, for the diagnosis of this disease. As noted above, in this work out of 46 reactors to avian tuberculin 24 were proved to be positive for Johne's disease, while out of 14 non-reactors, suspected of Johne's infection on other grounds, 9 were found to be positive. With regard to Johnin there are a number of observations in the literature. Reference to some of these is given by Minett [1933, 1935] who has also reported on the value of a synthetic Johnin for the diagnosis of Johne's disease in cattle. In a series of 53 animals which had reacted to the double intradermal method of testing, 39 were proved to be infected on *post mortem* examination, while there was presumptive evidence of this infection, either from *post mortem* examination or from the history of the case, in eleven of the remaining 14. Repeat tests on a number of the animals in this series showed much variation in the degree of local response to Johnin, a result which was attributed to variations in the allergic state. A few other cattle which were at an advanced stage of the disease did not react.

The vaccination experiments in the Mukteswar herd were started with the possibility in mind that the disease would spread easily and that in this way the value of vaccination might be judged. Unfortunately, since the disease did not spread and the number of calves available for vaccination was small, no conclusion can be drawn as to the usefulness or otherwise of the method and the second object of the work in the experimental herd was therefore not achieved.

The question may be asked finally in what way the conditions of the present experiment differ from those cases in which the disease seems to spread without hindrance. The favourable conditions under which the animals in this experiment were maintained have already been referred to and it seems probable that under good farming conditions the disease spreads easily only (a) when there is a high level of infective material within a restricted space, (b) when the level of host susceptibility is high, as when there is a high proportion of very young animals. With the present herd an attempt has been made to estimate the chances at different times of healthy animals acquiring infection by preparing a list of (a) the number of infected animals in January and July in each year and their status as regards clinical or latent infection and (b) the number of healthy or presumably healthy contacts and their age on the same dates. The ratio of (a) to (b) would clearly be a means of judging

the chances of spread at different stages of the experiment. The actual results of this enquiry are not detailed here but it is strongly suggested that the danger of infection spreading could at no time have been great, in view of the high average age of the contacts and the paucity of animals suffering from the disease in clinical form. Under these circumstances some 75 per cent of the animals at the most susceptible age escaped infection. The results of the Mukteswar experiment are in general accord with those of Hagan and Zeissig [1933] who found in their six years' experience of a herd experimentally infected with Johne's disease that even under conditions of severe exposure a considerable number of animals failed to contract the disease naturally.

#### SUMMARY

An account is given of a herd of cattle maintained at Mukteswar for a period of 10 years to determine the chances of Johne's disease spreading amongst them. Although a number of naturally infected adult animals died of the disease and although some 25 per cent of the calves born in the herd also became infected and died, there was no general spread of infection under the conditions there prevailing and at the termination of the experiment Johnin testing of the whole herd and *post mortem* examination of 6 animals selected at random indicated that the disease had disappeared.

It seems, therefore, that in practice there is no great danger of Johne's disease becoming established in a well-managed herd, unless there is a high initial level of infection in a restricted space, and a relatively large proportion of highly susceptible, i.e. very young animals, are present.

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## APPENDIX I

*History in brief of animals belonging to the experimental herd (showing the age at entry, duration of stay in herd and fate)*

- (1) C. Bihar. 4 yr. Aug. 29—May 31. Clinical case. Rect. sm. + + +. J. d. +.
- (2) C. Bihar. 5 yr. Aug. 29—Feb. 30. Clinical case. Rect. sm. + + +. J. d. +.
- (3) C. Bihar. 3 yr. Aug. 29—Jan. 30. Clinical case. Rect. sm. + + +. J. d. +.
- (4) C. Bihar. 10 yr. July 30—June 36. July 30. Bowel wash +. May 36 faeces +. J. d. +.
- (5) C. Bihar. 5 yr. July 30—Feb. 38. July 30. Bowel wash +. J. d. —. T. B. +.
- (6) C. Bihar. 11 yr. July 30—Feb. 31. July 30. Bowel wash +. J. d. —.
- (7) C. Bihar. 9 yr. July 30—April 36. July 30. Bowel wash +. J. d. +.
- (8) C. Bihar. 10 yr. July 30—June 37. July 30. Bowel wash +. J. d. +.
- (9) C. Bihar. 10 yr. July 30—Sep. 32. July 30. Bowel wash +. J. d. —.
- (10) C. Bihar. 7 yr. July 30—Nov. 30. July 30. Bowel wash +. J. d. +.
- (11) H. C. b. June 30 of C. 10 (9). D. Dec. 32. J. d. +.
- (12) Bk. Bihar. 10 yr. July 30—June 31. July 30. Bowel wash +. Sep. 30. Rect. sm. +. Dest. J. d. —. T. B. +.
- (13) Bk. Bihar. 12 yr. July 30—June 31. July 30. Bowel wash +. Dest. J. d. —. T. B. +.
- (14) C. Central Provinces. 3 yr. July 30—Jan. 39. Oct. 30. Rect. sm. +. May 36 faeces +. J. d. —.
- (15) C. Assam. 9 yr. July 37—Mar. 38. J. d. —.
- (16) C. Assam. 3 yr. July 37—June 38. J. d. —.
- (17) B. Assam. 7 yr. July 37—Sep. 37. Dest. J. d. +.
- (18) Bk. Assam. 5 yr. July 37—Sep. 37. Dest. J. d. —.
- (19) B. Assam. 4 yr. July 37—Sep. 37. Dest. J. d. +.
- (20) Bk. Assam. 7 yr. July 37—Sep. 37. Dest. J. d. —.
- (21) B. Assam. 1 yr. July 37—May 39. Dest. J. d. —.
- (22) B. Assam. 4 yr. Sep. 37—Mar. 39. D. J. d. +.
- (23) B. Assam. 1 yr. Sep. 37—April 39. Dest. J. d. —.
- (24) B. Assam. 1 yr. Sep. 37—April 39. Dest. J. d. —.
- (25) B. Assam. 1 yr. Sep. 37—April 39. Dest. J. d. —.
- (26) B. Assam. 1 yr. Sep. 37—April 39. Dest. J. d. —.
- (27) H. C. b. Mar. 29 Dairy Feb. 30 contact infected animals. Aug. 30 Rect. sm. +. D. Jan. 31. J. d. +.
- (28) C. b. Oct. 28 Dairy Jan. 32 transferred to J. herd being suspicious. Feb. 37 faeces +. D. Feb. 37. J. d. +.
- (29) C. b. Dec. 24 Dairy June 32 bowel wash +. Transferred to J. herd. D. June 32. J. d. +.
- (30) C. b. July 28 Dairy June 32 bowel wash +. Transferred to J. herd. D. Aug. 33. J. d. +.
- (31) C. b. Dec. 24 Dairy Oct. 32 faeces +. Transferred to J. herd. D. June 34. J. d. +.
- (32) C. b. Dec. 27 Dairy July 33 transferred to J. herd being suspicious. D. Sept. 33. J. d. +.
- (33) C. b. April 26 Dairy Sept. 33 faeces +. Transferred to J. herd. D. Oct. 33. J. d. +.
- (34) C. b. Feb. 32 Dairy April 34 bowel wash +. Transferred to J. herd. July 34 faeces +. D. Jan. 35. J. d. +.
- (35) C. b. April 32 Dairy May and June 36 Rect. sm. +. Transferred to J. herd. D. Aug. 36. J. d. +.
- (36) C. b. Jan. 32 Dairy June 36 faeces +. Transferred to J. herd. D. Sep. 36. J. d. +.
- (37) C. b. Aug. 25 Dairy Aug. 36 faeces +. Transferred to J. herd. D. Sep. 36. J. d. +.

APPENDIX 1—*contd*

- (38) C. b. Jan. 32 Dairy July 36 faeces +. Transferred to J. herd. D. Sep. 36. J. d. —.
- (39) B. b. Mar. 26 Dairy Sep. 30 Rect. sm. + + +. Transferred to J. herd. Dest. Nov. 30. J. d. +.
- (40) Bk. 8 yr. Dairy May—July 38. Faeces +. D. J. d. +.
- (41) Bk. 7 yr. Dairy May—July 39. Dest. J. d. —.
- (42) B. b. Sep. 28 Dairy July 31 transferred to J. herd. Aug. 33. Dest. J. d. —.
- (43) B. b. Sep. 27 Dairy Apr. 31 transferred to J. herd as stud bull. July 34 vaccinated. Dest. May 36. J. d. —.
- (44) B. 5 yr. (Dairy—purchased as a hill bull). July 31 artificially infected with J. culture intravenously. July 33 Dest. J. d. —.
- (45) Bk. b. Mar. 33 Dairy Vaccinated Sep. 33 and placed in J. herd. Dest. April 35. J. d. —.
- (46) H. b. Dec. 30 of C. 5 (61). D. Feb. 36. J. d. +.
- (47) H. b. Nov. 32 of C. 4 (4). Jan. 37 faeces + +. D. Aug. 37. J. d. +.
- (48) H. b. Jan. 33 of C. 5 (61). May 36 faeces + +. D. May 36. J. d. +.
- (49) H. b. July 33 of C. 31 (69). D. Jan. 39. J. d. +.
- (50) H. b. Apr. 32 of C. 10 (9). D. Apr. 36. J. d. —.
- (51) H. b. Jan. 32 of C. 20 (28). D. Oct. 34. J. d. —.
- (52) H. b. Apr. 35 of C. 4 (4). Vaccinated at birth. D. Nov. 37. J. d. —.
- (53) B. C. b. Aug. 29 of C. 3 (3). Rect. sm. +. D. May 31. J. d. +.
- (54) B. C. b. Sep. 30 of C. 6 (5). Vaccinated June 32. Rect. sm. +. D. Jun. 32. J. d. +.
- (55) B. C. b. Feb. 35 of C. 20 (28). D. Mar. 39. J. d. +.
- (56) B. C. b. Apr. 32 of C. 6 (5). Vaccinated June 37. Faeces + +. D. Jan. 39. J. d. +.
- (57) B. b. Mar. 34 of C. 6 (5). Vaccinated Apr. 35. Dest. J. d. +.
- (58) B. b. Oct. 30 of C. 7 (6). Dest. Aug. 34. J. d. —. T. B. +.
- (59) B. b. Aug. 33 of C. 11 (62). Dest. July 39. J. d. —.
- (60) Bk. b. Dec. 30 of C. 8 (7). Vaccinated. Dest. July 39. J. d. —.
- (61) C. Bihar. 3 yr. July 30 Bowel wash +. Still alive.
- (62) C. Bihar. 3 yr. July 30 Bowel wash +. Still alive.
- (63) C. Assam. 9 yr. Received July 37. Still alive.
- (64) C. Assam. 4 yr. Received July 37. Still alive.
- (65) B. Assam. 5 yr. Received July 37. Still alive.
- (66) B. Assam. 5 yr. Received Sep. 37. Still alive.
- (67) B. Assam. 5 yr. Received Sep. 37. Dest. May 40. J. d. —.
- (68) B. Assam. 3 yr. Received Sep. 37. Mar. 39 Faeces +. Dest. May 40. J. d. —.
- (69) C. b. Apr. 28 Dairy. Oct. 30 transferred to J. herd. Dest. Jun. 40. J. d. —.
- (70) C. 10 yr. Dairy. Sep. 36 transferred to J. herd. Dest. June 40. J. d. —.
- (71) C. b. Nov. 29 Dairy July 31 transferred to J. herd. Still alive.
- (72) C. b. Aug. 31 Dairy. Vaccinated June 32. Contact with infected herd. Still alive.
- (73) C. b. Jan. 31 of C. 4 (4). Still alive.
- (74) H. b. Jan. 35 of C. 31 (69). Still alive.
- (75) H. b. Jan. 37 of C. 31 (69). Still alive.
- (76) H. b. July 37 of C. 50 (81). Dest. May 40. J. d. —.
- (77) H. b. July 34 of C. 11 (62). Still alive. (Died after close of experiment. Body emaciated but no lesions of J. dis.).
- (78) H. C. b. Nov. 38 of C. 34 (72). Still alive.
- (79) H. C. b. Sep. 39 of C. 15 (63). Still alive.
- (80) C. b. Dec. 32 of C. 9 (8). Vaccinated. Still alive.
- (81) C. b. May 32 of C. 11 (62). Vaccinated. Still alive.
- (82) C. b. Aug. 33 of C. 20 (28). Vaccinated. Still alive.
- (83) H. b. Feb. 35 of C. 33 (71). Vaccinated. Still alive.
- (84) H. b. Mar. 36 of C. 42 (73). Vaccinated. Still alive.
- (85) H. C. b. Feb. 39 of C. 32 (70). Vaccinated. Still alive.
- (86) H. C. b. July 39 of C. 50 (81). Vaccinated. Still alive.



- (87) B. b. Jan. 35 of C. 5 (61). Still alive.  
 (88) B. C. b. Jun. 38 of C. 51 (82). Dest. July 40. J. d. —.  
 (89) B. C. b. July 39 of C. 31 (69). Still alive.  
 (90) B. b. Jan. 37 of C. 9 (8). Vaccinated. Still alive.  
 (91) B. b. July 37 of C. 5 (61). Vaccinated. Still alive.  
 (92) B. C. b. Mar. 39 of C. 33 (71). Vaccinated. Still alive.  
 (93) B. C. b. Sep. 39 of C. 49 (80). Vaccinated. Dest. May 40. J. d. —.  
 (94) H. C. b. Dec. 30 of C. 9 (8). Vaccinated. Oct. 32 Faeces +. D. Dec. 32. J. d.  
 +.  
 (95) H. C. b. July 37 of C. 11 (62). D. July 39. J. d. +.

Rect. sm. + = Rectal smear positive for Johne's bacilli.

J. d. + = Johne's disease confirmed post-mortem.

b. = Born.

Dairy = Mukteswar Dairy.

D = Died.

Dest. = Destroyed.

yr. = Year.

C. = Cow.

H. C. = Heifer calf.

Bk. = Bullock.

B. C. = Bull calf.

B. = Bull.

Only positive results of rectal smear, bowel washing and faeces examinations are shown.

## APPENDIX 2 (a)

*Results of tests with ordinary and avian tuberculin. (Animals with serial Nos. 1 to 60)*

Serial No.	Animal	Date of test	Result with		Diagnosis <i>post mortem</i>
			Avian tuberculin	Ordinary tuberculin	
1	C.	May 29 . .	+	—	J. d. +.
		Dec. 29 . .	—	—	
		Apr. 30 . .	—	—	
		Aug. 30 . .	—	—	
2	C.	May 29 . .	+	—	J. d. +.
		Dec. 29 . .	+	—	
3	C.	May 29 . .	+	—	J. d. +.
		Dec. 29 . .	+	—	
4	C.	Apr. 30 . .	+	—	J. d. +
		Aug. 30 . .	+	—	
		Dec. 31 . .	±	—	
		Jun. 33 . .	—	—	
		Jun. 34 . .	+	+	
		Dec. 34 . .	..	±	
		Sep. 35 . .	—	—	
5	C.	Apr. 30 . .	+	—	J. d. —. T. B. +.
		Aug. 30 . .	—	—	
		Jun. 33 . .	+	—	
		Jun. 34 . .	±	±	
6	C.	May 30 . .	+	—	J. d. —.
		Aug. 30 . .	±	—	
7	C.	July 30 . .	+	—	J. d. +.
		Aug. 30 . .	+	—	
		Nov. 31 . .	±	—	
		Jun. 33 . .	±	—	
		Jun. 34 . .	—	—	
8	C.	Apr. 30 . .	+	—	J. d. +.
		Aug. 30 . .	+	—	
		Nov. 31 . .	±	—	
		Dec. 31 . .	±	—	
		Jun. 33 . .	+	—	
		Jun. 34 . .	+	—	
		Jun. 35 . .	—	—	
		Jun. 36 . .	—	—	
9	C.	Apr. 30 . .	+	—	J. d. —.
		Aug. 30 . .	+	—	

Serial No.	Animal	Date of test	Result with		Diagnosis <i>post mortem</i>
			Avian tuberculin	Ordinary tuberculin	
10	C.	Apr. 30 . .	+	—	J. d. +.
		Aug. 30 . .	+	—	
11	H. C.	Aug. 30 . .	—	—	J. d. +.
12	Bk.	Apr. 30 . .	±	—	J. d. —. T.B. +. (Dest.)
		Aug. 30 . .	+	—	
		Jun. 31 . .	+	+	
13	Bk.	Apr. 30 . .	±	—	J. d. —. T. B. +. (Dest.)
		Aug. 30 . .	+	—	
		Jun. 31 . .	+	+	
14	C.	Oct. 30 . .	+	—	J. d. —. T. B. —.
		Jun. 31 . .	+	+	
		Jun. 32 . .	—	+	
		Sep. 32 . .	+	+	
		May 33 . .	+	+	
		Jun. 33 . .	—	—	
		Jun. 34 . .	±	—	
		Aug. 35 . .	—	—	
15	C.	Feb. 36 . .	+	—	J. d. —.
		Jun. 36 . .	+	—	
		Oct. 36 . .	+	—	
		Jan. 37 . .	±	—	
		Aug. 37 . .	±	—	
16	C.	July 36 . .	+	—	J. d. —.
		July 37 . .	—	—	
		Aug. 37 . .	—	—	
17	B.	Sep. 36 . .	+	—	J. d. +. (Dest.)
		Aug. 37 . .	—	—	
18	B.	July 36 . .	+	—	J. d. —. (Dest.)
		Jan. 37 . .	+	—	
		Aug. 37 . .	±	—	
19	B.	Sep. 36 . .	—	—	J. d. +. (Dest.)
		Aug. 37 . .	±	—	
20	B.	Apr. 35 . .	—	—	J. d. —. (Dest.)
		Feb. 36 . .	—	—	
		Sep. 36 . .	+	—	
		Jan. 37 . .	+	—	
		Aug. 37 . .	±	—	
21	B.	Aug. 37 . .	—	—	J. d. —. (Dest.)

Serial No.	Animal	Date of test	Result with		Diagnosis <i>post mortem</i>
			Avian tuberculin	Ordinary tuberculin	
22	B	Mar. 37 . .	+	—	J. d. +.
		Sep. 37 . .	±	—	
23	B.	Mar. 37 . .	+	—	J. d. —. (Dest.)
		Sep. 37 . .	+	—	
		Mar. 39 . .	—	—	
24	B.	Mar. 37 . .	+	—	J. d. —. (Dest.)
		Sep. 37 . .	—	—	
		Mar. 39 . .	—	—	
25	B.	Mar. 37 . .	+	—	J. d. —. (Dest.)
		Sep. 37 . .	±	—	
		Mar. 39 . .	—	—	
26	B.	Mar. 37 . .	+	—	J. d. —. (Dest.)
		Sep. 37 . .	±	—	
27	H. C.	Aug. 30 . .	+	—	J. d. +.
28	C.	Jun. 31 . .	±	—	J. d. +. T. B. —.
		Oct. 31 . .	±	—	
		Dec. 31 . .	±	—	
		Jun. 33 . .	+	+	
		Jun. 34 . .	+	+	
		Dec. 34 . .	..	+	
29	C.	Oct. 30 . .	±	±	J. d. +.
		Nov. 30 . .	..	—	
		Mar. 32 . .	±	—	
30	C.	Oct. 30 . .	+	+	J. d. +.
		Jan. 32 . .	±	—	
		Jun. 33 . .	—	—	
31	C.	Oct. 32 . .	—	—	J. d. +.
		Jun. 33 . .	—	—	

J. d. +. = Johne's disease confirmed post-mortem.

J. d. —. = Johne's disease not confirmed post-mortem.

T. B. +. = Tuberculosis confirmed post-mortem.

T. B. —. = Tuberculosis not confirmed post-mortem.

± = Suspicious probably negative.

+ = Suspicious.

+ = Positive to test.

C. = Cow.

H. C. = Heifer calf.

Bk. = Bullock.

B. C. = Bull calf.

B. = Bull.

H. = Heifer.

Unless otherwise stated the animal died.



Serial No.	Animal	Date of test	Result with		Diagnosis <i>post mortem</i>
			Avian tuberculin	Ordinary tuberculin	
32	C.	Jun. 33 . .	—	—	J. d. +.
33	C.	Oct. 30 . .	+	—	J. d. +.
		May 31 . .	—	—	
		Jun. 32 . .	—	—	
		Jun. 33 . .	—	—	
34	C.	Nov. 33 . .	±	—	J. d. +.
		Jun. 34 . .	—	—	
35	C.	Jun. 33 . .	—	—	J. d. +.
		Jun. 34 . .	—	—	
		Jun. 35 . .	—	—	
36	C.	Jun. 33 . .	—	—	J. d. +.
		Jun. 34 . .	±	—	
		Dec. 34 . .	±	—	
		May 35 . .	—	—	
		July 36 . .	—	—	
37	C.	Oct. 30 . .	+	—	J. d. +.
		Jun. 36 . .	—	—	
38	C.	Jun. 33 . .	—	—	J. d. —.
		Jun. 34 . .	±	—	
		Dec. 34 . .	—	—	
		May 35 . .	—	—	
39	B.	Oct. 30 . .	+	—	J. d. +. (Dest.)
40	Bk.	Mar. 39 . .	+	—	J. d. +.
41	Bk.	Mar. 39 . .	+	—	J. d. —. (Dest.)
42	B.	Jun. 31 . .	+	—	J. d. —. (Dest.)
		Dec. 31 . .	—	—	
		Jun. 32 . .	±	—	
		Jun. 33 . .	±	—	
43	B.	Jun. 33 . .	—	—	J. d. —. (Dest.)
		Jun. 34 . .	—	—	
44	B.	July 31 . .	—	—	J. d. —. (Dest.)
		Sep. 31 . .	+	—	
		Nov. 31 . .	+	—	
		Jun. 33 . .	+	—	
45	B.	Jun. 34 . .	+	+	J. d. —. T. B. —. (Dest.)
		Dec. 34 . .	..	+	

Serial No.	Animal	Date of test	Result with		Diagnosis
			Avian tuberculin	Ordinary tuberculin	
46	H.	Jun. 33 . .	+	—	J. d. +.
		Jun. 34 . .	+	—	
47	H.	Jun. 33 . .	—	—	J. d. +.
		Jun. 34 . .	—	—	
		Sep. 35 . .	—	—	
48	H.	Jun. 33 . .	—	—	J. d. +.
		Jun. 34 . .	—	—	
		Jun. 35 . .	—	—	
49	H.	Jun. 34 . .	—	—	J. d. +.
50	H.	Jun. 33 . .	—	—	J. d. —.
		Jun. 34 . .	—	—	
51	H.	Jun. 33 . .	—	—	J. d. —.
		Jun. 34 . .	±	—	
52	H.	Jun. 35 . .	—	—	J. d. —.
		Jun. 36 . .	+	—	
53	B. C.	Aug. 30 . .	+	—	J. d. +.
		Feb. 31 . .	+	—	
54	B. C.	Feb. 31 . .	+	—	J. d. +.
55	B.	Jun. 35 . .	—	—	J. d. +.
		Jun. 36 . .	—	—	
56	B. C.	Jun. 33 . .	—	—	J. d. +.
		Jun. 34 . .	—	—	
57	B.	Jun. 34 . .	+	+	J. d. +. (Dest.)
		Dec. 34 . .	..	—	
58	B.	Jun. 33 . .	+	+	J. d. —. T. B. +. (Dest.)
59	B.	Jun. 34 . .	—	—	J. d. —. (Dest.)
60	Bk.	Jun. 33 . .	—	—	J. d. —. (Dest.)
		Jun. 34 . .	—	—	

## APPENDIX 2 (b)

Results of tests with ordinary and avian tuberculin and with synthetic tuberculin and Johnin  
(Animals with Serial Nos. 61 to 95)

Serial No.	Animal	Date of test	Avian tuberculin	Ordinary tuberculin	Synthetic tuberculin	Synthetic Johnin	Diagnosis post mortem	Remarks
61	C.	Apr. 30	+	—				
		Aug. 30	+	—				
		Dec. 31	±	—				
		Jun. 33	—	—				
		Apr. 40	..	..	—	—	..	Still alive
62	C.	Apr. 30	+	—				
		Aug. 30	+	—				
		Jun. 33	+	+				
		Jun. 34	±	±				
		Apr. 40	..	..	—	—	..	Still alive
63	C.	Feb. 36	+	—				
		Oct. 36	+	—				
		Aug. 37	±	—				
		Apr. 40	..	..	—	—	..	Still alive
64	C.	Aug. 36	+	—				
		Aug. 37	±	—				
		Apr. 40	..	..	—	—	..	Still alive
65	B.	Feb. 36	—	—				
		Sep. 36	+	—				
		Jan. 37	±	—				
		Aug. 37	±	—				
		Apr. 40	..	..	—	—	..	Still alive
66	B.	Aug. 36	+	—				
		Sep. 37	+	—				
		Apr. 40	..	..	—	—	..	Still alive
67	B.	Mar. 37	+	—				
		Sep. 37	—	—				
		Apr. 40	..	..	—	—	J. d. —.	(Dest.)
68	B.	Mar. 37	+	—				
		Sep. 37	±	—				
		Mar. 39	+	—				
		Apr. 40	..	..	—	—	J. d. —.	(Dest.)
69	C.	Oct. 30	+	—				
		Jun. 33	—	—				
		Jun. 34	—	—				
		Apr. 40	..	..	—	—	J. d. —.	(Dest.)

Animal	Date of test	Avian tuber- culin	Ordinary tuber- culin	Synth- etic tuber- culin	Synth- etic Joh- nin	Diagnosis	Remarks
C.	July 29 .	—	—				
	Oct. 30 .	—	—				
	Jun. 32 .	—	—				
	Jun. 33 .	—	—				
	Aug. 36 .	+	—				
	Apr. 40 .	..	..	—	—	J. d. —.	(Dest.)
C.	Jun. 31 .	+	—				
	Dec. 31 .	±	—				
	Jan. 32 .	+	—				
	Jun. 33 .	—	—				
	Jun. 34 .	—	—				
	Apr. 40 .	..	..	—	—	..	Still alive
C.	Jun. 32 .	+	—				
	Jun. 33 .	±	—				Vaccinat- ed
	Jun. 34 .	—	—				
	Apr. 40 .	..	..	—	—	..	Still alive
C.	Jun. 33 .	—	—				
	Jun. 34 .	—	—				
	Apr. 40 .	..	..	—	—	..	Still alive
H.	Jun. 35 .	—	—				
	Jun. 36 .	—	—				
	Apr. 40 .	..	..	—	—	..	Still alive
H.	Apr. 40 .	..	..	—	—	..	Still alive
H.	Apr. 40 .	..	..	—	—	J. d. —.	(Dest.)
H.	Sep. 35 .	—	—				
	Jun. 36 .	—	—				
	Apr. 40 .	..	..	—	—	..	Still alive
H. C.	Apr. 40 .	..	..	—	—	..	Still alive
H. C.	Apr. 40 .	..	..	—	—		Still alive
C.	Jun. 33 .	—	—				
	Jun. 34 .	±	—				Vaccinat- ed
	Apr. 40 .	..	..	—	—	..	Still alive
C.	Jun. 33 .	±	±				
	Jun. 34 .	—	—				Vaccinat- ed
	Jun. 35 .	—	—				
	Jun. 36 .	—	—				
	Apr. 40 .	..	..	—	—	..	Still alive
C.	Jun. 34 .	+	—				Vaccinat- ed



Serial No.	Animal	Date of test	Avian tuber- culin	Ordinary tuber- culin	Synth- etic tuber- culin	Synth- etic Joh- nin	Diagnosis	Remarks
83	H.	Apr. 40	..	..	—	±	..	Still alive
		Jun. 35	—	—				Vaccinat- ed
		Jun. 36	—	—				Still alive
		Apr. 40	..	..	—	—	..	
84	H.	Jun. 36	±	—				Vaccinat- ed
		Apr. 40	..	..	—	+	..	Still alive
85	H. C.	Apr. 40	..	..	—	±	..	Vaccinat- ed
								Still alive
86	H. C.	Apr. 40	..	..	+	+	..	Vaccinat- ed
								Still alive
87	B.	Jun. 35	—	—				
		Jun. 36	—	—				
		Apr. 40	..	..	—	—	..	Still alive
88	B. C.	Apr. 40	..	..	—	—	J. d.—.(Dest.).	
89	B. C.	Apr. 40	..	..	—	—	..	Still alive
90	B.	Apr. 40	..	..	+	+	..	Vaccinat- ed
								Still alive
91	B.	Apr. 40	..	..	—	+	..	Vaccinat- ed
								Still alive
92	B.	Apr. 40	..	..	+	+	..	Vaccinat- ed
								Still alive
93	B. C.	Apr. 40	..	..	+	+	J. d.—.(Dest.).	Vaccinat- ed
94	H. C.	..	..	..	..	..	J. d. +.	
95	H. C.	..	..	..	..	..	J. d. +.	

## APPENDIX 3

*Vaccination Results*

Year	No. of animals	Found infected with Johne's disease	Johne's infection not found	Still living	Remarks
1930-33 .	8 vaccinated .	3	2	3	Bacilli in saline used for vaccination
	8 controls .	4	*3	1	
1934-40 .	14 vaccinated .	1	4	9	Bacilli in oil and sand used for vaccination
	13 controls .	2	2	9	

\* One was tubercular

# MULTIPLICATION OF *B. ANTHRACIS* AND *CL. CHAUVÆI* IN SOIL AND IN WATER

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[AMONG the many interesting and important problems concerning animal disease in warm countries, the influence of 'climate' is one of the most outstanding. Much work has been done on this subject on the medical side by epidemiologists and others and in course of time we hope to extend knowledge of the subject as it affects the domesticated animals of India. By way of illustration, we would mention that exact information is wanted on the incidence of certain diseases in different parts of the country and at different seasons and this we are now collecting with the help of the local veterinary authorities. We trust that facts, which may be forthcoming in this and similar enquiries, will help towards a better understanding of methods of prevention and control. In the realm of infectious diseases, we are concerned, of course, with parasite and host and the conditions which may facilitate or hinder effective contact between them. In this first paper, we deal with a problem relating to the parasite, selecting as examples the organisms of anthrax and blackquarter—F.C.M.]

## LITERATURE

### *Anthrax*

A common view, early expressed by Robert Koch, is that the anthrax bacillus is an accidental parasite, which can live and multiply as a saprophyte in water containing plant residues. Anthrax, in fact, is often referred to as a soil disease. Circumstantial evidence for this is that the disease in warmer countries is frequent at certain seasons in areas where the soil is wet or marshy or which have been subjected to recent flooding. In India, anthrax tends to break out with the onset of the rainy season.

Mollet [1913] gives a number of references to the older literature on the subject. The position is also summarized among others by Oppermann [1906] and by Szpilman [1914]. One of the earliest workers on the subject was Schrakamp [1884], who came to the conclusion that the anthrax bacillus can undergo its whole developmental cycle in soil. It should be noted, however,

that in his experiments urine, blood or hay infusion were added to the soil, that all the materials were sterilized by heat and that the methods used, being based merely on microscopical appearances, would now-a-days be considered hardly sufficient. While his conclusions may have been correct in the circumstances, his work certainly does not support the contention that the organism can multiply in soil under natural conditions.

In a publication from the United States Department of Agriculture, written by Salmon and Theobald Smith [1904], it is said, 'Meteorological conditions also have an important share in determining the severity of the disease. On these tracts subject to inundations in spring, a very hot, dry summer is apt to cause a severe outbreak. The relation which the bacillus bears to these conditions is not positively known. It may be that during and immediately after inundations or in stagnant water the bacilli find enough nourishment in the water here and there to multiply and produce an abundant crop of spores.' Milks [1910] thought that his laboratory experiments indicated that anthrax bacteria would grow in low, swampy places or in any place where there was sufficient moisture. In a popular bulletin on anthrax written by Higgins [1916] and issued by the Department of Agriculture, Ottawa, the following statement appears: 'Our experiments would also indicate the probability that the germ is not only capable of remaining alive in soil but that it can also reproduce itself where conditions are favourable. All soils, however, would not lend themselves with equal facility to the vegetation of the anthrax organism. A suitable soil must be slightly alkaline if marked growth is to take place. We have found that a decoction of leaves is too acid to permit the growth of the bacilli. When, however, such a decoction is tempered with an alkaline soil, growth might take place under favourable temperature conditions.'

Szpilman [1914] states that the disease is indigenous in certain regions (so-called anthrax districts) which are of a marshy humus-rich nature over an impervious subsoil. But it is pointed out that it may also occur on other soil formations, e.g. chalk or clay. In Pomerania, anthrax occurs almost exclusively on peat pastures with lime subsoil or on heavy loam, close to river beds [Stampa, 1935]. Pokschischewsky and Golowin [1933] note the usual belief that favourable conditions for the development of anthrax are found on soils that are rich in organic matter and sufficiently damp. They attempted to obtain support for this contention by periodic recording of the pH, moisture content and temperature of the soil from permanently infected areas, as well as by bacteriological examination of the soil for anthrax. Few details are given and the claims of the authors are largely hypothetical. Burow [1912] in Germany could find no correlation between anthrax outbreaks and the composition of the soil. He considers that in the warm season the anthrax bacillus, thanks to nutritive substances fortuitously offered to it, finds in the soil an opportunity for better 'development'. The surface layers in particular have to be considered.

Writing of the disease in South Africa, Kehoe [1917] states that there are special districts where the disease appears to have been unusually prevalent. The author also refers to Hutcheon, who believed that the prevalence of anthrax in South Africa in summer and autumn was to be explained by



the temperature requirements for the growth and multiplication of anthrax bacilli. Viljoen, Curson and Fourie [1928] in South Africa remark that it is unknown 'whether the anthrax organism can persist only in the spore form or whether the spores can vegetate under certain favourable conditions and in this way increase in numbers'. This important point 'has never been settled by direct experimental evidence'. 'In some parts of Europe, the organism is believed to be a strict obligatory parasite but this belief is based purely on circumstantial evidence'. In South Africa, the disease is prevalent both on wet and on dry pastures so that the moisture content of the soil appears to have little significance. M'Fadyean [1898] states that, although the bacillus frequently finds outside the body all the conditions essential for its multiplication, in temperate climates growth is not likely to make much headway owing to the temperature not being favourable, to competition with more rapidly growing organisms and to desiccation.

With regard more specifically to water, Frankland and Ward [1893], in their Second Report to the Water Research Committee of the Royal Society, summarize the work of some previous investigators on the viability of anthrax bacteria in water and state that anthrax spores in sterile or unsterilized water remain alive for many months at ordinary or low temperatures but are slowly destroyed at 35°C. They themselves found that spores persisted in the living state for many months and in much larger numbers in sterile river or lake water than in these waters in the unsterilized state, irrespective of whether the waters were preserved at 18°-20°C. or at 4°-9°C. In the unsterilized waters, there was a somewhat rapid and, up to a point, continuous decline in the number of living spores, so that after the lapse of a few days they were often no longer recognizable; an unfavourable effect of temperature (18°-20°C.) upon survival being particularly noticeable with lake water. Direct (winter) sunlight had a marked and direct bactericidal effect—due not to the heat rays but to the light rays—both in sterile and in natural water, the spores added being killed, often within 84 hours. They also found that, while the organism does not propagate in sterile distilled water, it can multiply extensively in sterilized city sewage. They were unable to show that *B. anthracis* multiplies to any considerable extent in freshly collected river water, sterilized by filtration or by heat or in the unsterilized state, unless appreciable quantities of organic food materials are added and the temperature is above 12°C. Apparently, if anthrax bacteria in the vegetative state are introduced, they die off within the first day or two or, if conditions are favourable, they sporulate. All told, there was nothing to support the view that *B. anthracis* can live and multiply as does a water bacterium in ordinary water. Szasz [1914] also, in referring to anthrax arising from pond drinking water, considers that it is not due to actual multiplication of the organism in the water or its muddy sediment.

As to the viability of anthrax spores under natural conditions, there is no dispute. Hastings [1923] has recently mentioned the high resistance of anthrax spores from a natural habitat, as compared with spores from artificial media; in this matter he is in agreement with Oppermann [1906]. In a sample of naturally infected pond water, Hastings found spores to be alive after 18½ years.

From a perusal of veterinary literature, it may be concluded that there is much uncertainty as to whether pathogenic organisms which find their way into the soil actually multiply therein. It is well known, on the other hand, that many of the common soil bacteria have an antagonistic action upon bacteria, pathogenic or otherwise. A familiar and classical example is the inhibitory effect of 'bacillus pyocyaneus' upon the anthrax bacillus but many other bacteria have a similar action. For instance, Isabolinski and Sobolewa [1934] have recently emphasized that *Bact. coli* is antagonistic to *B. anthracis* both *in vivo* and *in vitro*; when both are sown together in broth, *coli* alone grows. *Proteus vulgaris* and also mixtures of streptococci and staphylococci are likewise antagonistic. As Lewis [1929] and Waksman and Woodruff [1940] point out, a rich literature has now accumulated on the subject of bacterial antagonism and many efforts have been made to determine the properties and nature of the antagonistic substances produced.

#### *Blackquarter*

Blackquarter also is referred to as a soil disease and its prevalence in certain countries is stated to be similar, in many respects, to that of anthrax and to be related to particular types of soil. Thus, Hunziker [1927] states that in Switzerland outbreaks of blackquarter are related to the chalk content of the soil. Viljoen and Scheuber [1926], writing of blackquarter in South Africa, state that, although the disease occurs in all parts, it tends to be regionally distributed, being more prevalent in low-lying areas. There is no reason to believe that the disease in South Africa is connected with any special kind of soil but its occurrence is seasonal, being at its worst during the spring and early summer months and especially after heavy rains. The organism is generally believed to be a facultative parasite, capable of existing and multiplying in the soil of certain localities, but, as the authors state 'no experimental evidence to prove this contention or to show how long the organism can exist outside the animal body has ever been advanced'. Seddon and Edgar [1930] say that, although the soil of damp or marshy areas is usually regarded as the normal habitat of *Cl. chauvvei*, this opinion has not, apparently, been confirmed bacteriologically. They themselves have never isolated the organism from the soil of localities where blackquarter is enzootic. Probably the organism is confined to spots where the carcasses of animals dead of the disease have disintegrated.

It is not perhaps altogether out of place to refer here to some of the work which has been done on the behaviour of other pathogenic organisms in soil and water. Towards the end of the last century, a considerable amount of work was carried out on this subject and the position as regards water is thus summarized by Henry [1929], "Where the organic content remains at a high level, as in heavy pollution with sewage, the organisms that are particularly adapted to a parasitic existence in the bodies of animals and plants may survive for some time, but with dilution and oxidation they tend to die out and disappear, leaving only the ordinary harmless water bacteria to take their place". With regard to *Bact. typhosum* and the effect of temperature on its survival in water, Houston [1911] noted that after a week 14 per cent

of added organisms remained alive at 5°C., while at 10°C. only 0·07 per cent survived. In the case of soil, Martin [1897-98] found that *Bact. typhosum* would grow at 37°C. in moist sterile soil containing much organic matter and that some growth also occurred at 15°-19°C. In sandy or peaty, unmanured, sterile soil, there was no growth, even at 37°C. Mair [1908], on the other hand, could get no evidence of *Bact. typhosum* multiplying in either sterilized or unsterilized soil. In some samples, but not in all, the organism died out much more rapidly if the soil had been previously autoclaved, apparently owing to the production of bactericidal substances during sterilization. Harvey [1929] quoted the important observation of a urinary carrier who was caused to micturate on to a patch of soil in a dark hut; typhoid organisms could not be isolated from the soil much beyond 24 hours. With *V. cholerae*, there is stated to be no support for the view that the epidemic prevalence of the disease depends on the vitality of the organism outside the body; some natural waters for example are well known to be rapidly lethal. The subject is dealt with in a recent paper by Read, Singh, Seal and Bose [1939], to which reference may be made for further particulars.

The following remarks concern bacteria of importance in animal pathology. Organisms of the *Clostridium* group are regarded as primarily saprophytic in nature, living in the intestine of animals and proliferating also in decaying animal and vegetable matter. It is of interest to note that at least one species, viz. *Cl. botulinum*, is common in virgin soil and hence one might expect it to be capable of multiplying therein. Bull [1939] refers to the possibility of *Cl. welchii* type D growing in soil rich in organic matter and under favourable temperature and moisture conditions, and considers that in this possibility lies an explanation of outbreaks of ovine enterotoxaemia. Fildes [1929], writing of tetanus, says, 'It is generally assumed that in the soil the bacteria are present as spores, but the possibility of vegetation under certain circumstances has never been considered'. As to *Corynebacteria*, Carne [1932] found that *C. ovis* will grow readily at 37°C. in the autoclaved faeces of sheep which had been fed on certain diets. The author was 'not successful in carrying out experiments with unsterilized faeces'. Bull and Dickinson [1933] also believe that *C. ovis* may grow in soil and accumulated vegetable matter at seasons when the moisture conditions are favourable. There was no direct evidence of this and the reason for their belief was the ease with which soil from places where ovine caseous lymphadenitis is enzootic will sometimes infect guinea-pigs. The importance of soil reaction in respect of survival of *E. rhusiopathiae* has been pointed out by Hesse [1924], survival being considerably greater in alkaline than in acid soils.

#### TECHNICAL DETAILS

##### *Soil*

For most of the experiments, soil from the Institute inner area at Mukteswar was used. The bulk samples (A, B and C) were taken from a not obviously manured plot at depths varying from 3 in. to 12 in. For certain experiments, soil samples from the Imperial Agricultural Research Institute and from Madras were used. The nature and analysis of the soils used is shown in Table I.



The Mukteswar soils were passed first through a coarse sieve, then ground lightly and put through a 1 mm. sieve; the sieved soil was then air-dried and stored in jars at laboratory temperature. At time of use, 25-50 gm. were transferred to 125 c.c. bottles of 5 cm. diameter, water was added and the wool-plugged bottles autoclaved for 1-2 hours at 20-25 lb. pressure. Later in attempting to surmount the difficulty of sterilizing this amount of soil at one or two heatings in the small bottles, the soil was placed in thin layers in open beakers and flooded with water before autoclaving. After cooling, the wet soil was placed in the bottles and re-autoclaved. When cold, sterile water was added to give the required concentration and indiarubber corks were fitted to the bottles. (One of the major difficulties in this work has been to sterilize the soil effectively; our efforts were not always successful, even when 25 gm. amounts spread in thin (about 1 cm.) layers and well flooded with water were autoclaved). Tests were also made with natural (i.e. unsterilized) soil. At the conclusion of the experiment, the water content of each sample was taken as a check.

#### Water

A few experiments were made to see if *B. anthracis* and *Cl. chauvœi* would grow in tap water (500 c.c. in round flasks), sterilized or natural, over a thin layer of soil.

#### Seed

(a) The anthrax strain used had been isolated in Burma from an elephant and its virulence for sheep and guinea-pigs was established. It had apparently lost much of its virulence for hill cattle. In preparing the suspension to be added to the soil, the sporulated growth from an agar slant after 4 or 5 days at 37°C. was evenly suspended with the aid of glass beads in distilled water to such a concentration that a platinum loop disappeared from view at about 15 mm. below the surface. The viable count in such a suspension varied considerably, from 12 to 90 millions per c.c. but was usually between 20 and 40 millions. From this a suitable dilution was prepared, such that 1.0 c.c. of it added to and then thoroughly mixed with the soil was estimated to give 100-300 organisms, occasionally more, per gm. The actual count was, of course, estimated at the time of seeding. When samples were taken after incubation the soil was again thoroughly mixed. With very wet soils, this mixing was easy but it tended to be rather more difficult with pasty soils.

On a few occasions, heart blood from guinea-pigs freshly dead of anthrax was used for sowing the bottles.

(b) The *Cl. chauvœi* strain used was virulent for sheep and guinea-pigs. Its characters were checked for us by Mr V. R. Rajagopalan, as follows: subterminal spores swelling the rod, Claudius +, no blackening of chopped-meat or brain-liver medium, gelatin liquefied, no liquefaction of solid serum, no change in litmus milk, glucose, maltose, lactose and sucrose fermented and salicin and mannitol not fermented. The suspension to be used for seeding the soil was prepared from 3 or 4-day Robertson meat medium cultures, which at that time contained many spores. The organisms were washed once in distilled water and suspended in that fluid; the approximate total



count was estimated from breed smears and the actual viable count of the dilution used for the soil or water was obtained by culture in tubes of brain-liver medium [Haslam, 1920].

#### *Estimation of growth*

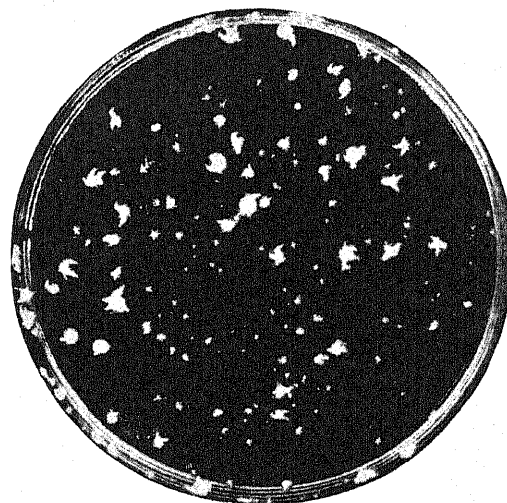
After incubation at temperatures of 20°C., 25°C., 30°C. and occasionally 37°C. for periods of 5 days and upwards the samples were examined for evidence of growth.

(a) Sterile soils which had been seeded with anthrax spores were plated in agar; natural soils were examined by inoculation of guinea-pigs or, occasionally mice. By means of a sterile spoon, the soil was thoroughly mixed with any supernatant water and a sample usually exceeding 4.0 gm. and often amounting to 6 or 7 gm., was removed from the bottle to a watch glass. As much soil as was reasonably possible was removed in order to minimize the sampling error. After further mixing on the watch glass, 4.0 gm. were transferred to 20 c.c. sterile saline (dil. *a*) and from this tenfold falling dilutions (dil. *b*, dil. *c*, etc.—1 c.c. to 9 c.c. saline) were made. Before removing 1.0 c.c. from dil. *a*, the soil was very well washed in the saline by repeated inversion of the tube and every effort made to keep the soil moving with the pipette and mixed as uniformly as possible, while 1 c.c. for transfer to dil. *b* was rapidly taken. Plates were sown from dil. *b*, *c*, etc., but not from dil. *a*, since the rapid sedimentation of most of the soil in this tube made the count of no absolute or relative value. The same technique was invariably followed and was carried out by the same operator, so that, it is believed, the counts on different samples of soil are reasonably comparable. The counts were made after 24 hours at 37°C. (The limitations of plating methods, as emphasized in the case of soil by Thornton [1922], are appreciated but we are not concerned with absolute numbers so much as with relative numbers. On several occasions, the soil samples have been thoroughly ground in a sterile mortar before suspending them in saline but no evidence could be found that the bacterial count was increased thereby).

As a rule, anthrax colonies are easily recognized; surface ones in particular present the characteristic ground-glass appearance with ragged edges or feathery outgrowths (Plate XXI). Deep colonies, though less developed, can likewise be recognized by their filamentous character. In the early stages of the work, a number of colonies were picked for identification by animal inoculation. Much difficulty was caused at times by spreading surface contamination and many plates had to be discarded for this reason.

With natural soils a similar procedure was followed, but 1.0 c.c. amounts of appropriate dilutions were inoculated subcutaneously to guinea-pigs or, in the earlier stages, to white mice. Mice, however, were not found to be uniformly susceptible and were therefore unsuitable.

(b) In the examination of soil for growth of *Cl. chauvœi*, the same technique was used both with sterilized and natural soil. After thorough mixing, amounts in excess of 4.0 gm. were removed to large sterile tubes and weighed. Sterile saline was then added at the rate of 20 c.c. to 4.0 gm., the soil well mixed and dilutions made with separate pipettes in sterile saline made as before, except that, for dil. *b*, 2 c.c. dil. *a* were mixed with



Characteristic surface and deep colonies  
of *B. anthracis* on agar



18 c.c. saline. 1.0 c.c. amounts were then seeded into tubes of brain-liver medium. After 24 and 48 hours at 37°C. those tubes showing gas were noted. After incubation, a microscopical examination was made from all the culture tubes and those sown from sterilized soil dilutions were also planted out on agar to detect any aerobic contamination. It was found that in the conditions of experiment gas production could usually be taken as an index of growth of *chauvæi* but results were only recorded as positive in this sense when gas production was associated with the presence of sporing organisms like *chauvæi*. A technique on similar lines was used with water.

#### GROWTH OF *B. ANTHRACIS* IN SOIL

##### A. Sterilized soils

It was necessary, in the first place, to find out whether the organism would multiply in sterilized soils, in spite of their artificial nature. If evidence of growth were to be obtained, it would then become necessary to ascertain suitable temperature and moisture conditions and whether growth could be improved by the addition of manurial substances. The problem of growth in natural (unsterilized) soils could then be studied.

Table II shows the results of experiments with a sample (B) of Mukteswar soil, in which the water content was estimated to vary from 0 to 40 per cent. The actual water contents, taken at the conclusion of the experiments, varied from 4 to 35 per cent, the tendency being for very dry soils to absorb some moisture during sterilization. After being seeded with organisms in numbers estimated in experiments 1 to 5 to vary from 50 to 315 per gm. of wet soil, the well-closed bottles were kept at 25°C. (usually) and counts made at intervals up to 35 days. It will be seen, not only that the anthrax bacillus is capable of multiplying in sterile soil at 25°C., but also that, as would be expected, the degree of multiplication bears a relationship to the water content. At water content up to 4 per cent, there was no evidence of growth; at 5.6 per cent it was slight; at 10.11 per cent it became very definite but was still not optimal; it was only when the amount of water reached 20 per cent or more that multiplication became at all extensive.

These results have some meaning only in respect of the particular soil in use, the important thing in general being that the soil should be thoroughly wet or muddy. In bottled soil in this state the distribution of water is not uniform, because the soil tends to become packed at the bottom leaving the upper layers water-logged. Soils not only vary enormously in their composition but their water-holding capacity also varies in accordance with the clay content, coarse or fine texture, etc. The amount of free water will likewise vary and it is largely upon this that bacterial proliferation will depend. In this connection, experiments were carried out with sample (B) of Mukteswar soil, sieved so as to give 'fine soil' with particles below 0.5 mm. diameter and 'coarse soil' with particles from 1.2 mm. diameter. When water to, say, 25 or 30 per cent of dry soil weight was added to each, the coarse soil became quite 'slushy', while the fine soil formed a stiff paste. The results given in Table II show that the anthrax bacillus grows well in the coarse soil but not in the fine, owing to the abundance of free water in the former.



It was not considered necessary to try to obtain any very accurate data as to the rate or extent of growth under the different conditions. Multiplication may go on up to 13 or 14 days at 25°C. but it is only occasionally that it is continued beyond that time. After 13-14 days, the number of colonies which develop tends to remain stationery; this, in conjunction with the observation that most of the organisms resist 60° or 70°C. for 30 minutes, indicates that they have passed into the spore phase. A glance at Table II will show that, under favourable moisture conditions, the number of organisms may increase by several thousandfold—in two cases by 25,000 and 30,000 times, representing a final count of 4.5 and 5.5 millions per gm. wet soil. Good growth also takes place when blood from guinea-pigs dead of anthrax is used as seed. There is, however, much variation in growth among the bottled soils kept under the same conditions and even in the absence of recognizable aerobic contamination.

A few attempts were made to demonstrate growth in parallel with the cultural results by inoculation of white mice and rats. This succeeded in some cases with mice but as previously remarked, the susceptibility of these animals is not uniform. As was expected, white rats were useless as test animals.

An endeavour was also made to determine the most favourable temperature range for the anthrax bacillus in soil. The results, shown in Table III, were somewhat erratic but two points were clear:—(i) that 15°-17°C. is too low; (ii) that good growths are obtainable at 25° and 30°C. There was a tendency for growth to be comparatively poor at 37°C. but this finding should be treated with reserve.

Experiments were conducted on soil to which certain 'manures' (horse dung, vegetable manure, ammonium sulphate, lime, superphosphate) were added. It was ascertained that the anthrax bacillus would grow in sterilized soil containing these substances individually or in mixture. Whether the organism grew better in the presence of some or all of these substances was not determined. The making of exhaustive observations on these points would have entailed much time and labour, which would have been scarcely justified by the results, especially as much difficulty was encountered in efficiently sterilizing soil containing the organic manures. One would expect, however, that such substances would assist growth and Erdman [1928], in studying the effect of various manurial treatments on the number of micro-organisms in soil, found that the addition of manure+lime+superphosphate brought the greatest increase in numbers. From the data in Table IV, it is seen that the anthrax bacillus grows in soil to which these substances severally have been added—in amounts larger no doubt than would be used in agricultural practice—though in some cases the results are erratic. The addition of lime appears to be especially advantageous, maximal growth occurring at 30°C. within 13 days.

*Growth in soils from other localities in India.*—It was of interest to ascertain whether the anthrax organism was capable of multiplying in sterilized soil, differing in composition from that at Mukteswar. For this purpose, 9 different types of soil were obtained from the Imperial Agricultural Research Institute, New Delhi, and 6 soils from the Agricultural College, Coimbatore, Madras.

The behaviour of the anthrax bacillus in these soils is shown in Table V. It is seen that growth takes place in all cases, except in very acid soils, where as would be expected, there is a tendency for growth to be inhibited. Naturally, the saturation capacity of these soils varied greatly but they were all brought to the water-logged state before being seeded. It may here be noted that, according to Koci [1934], the extreme pH limits for growth of the anthrax bacillus are 6.6 and 8.7, with an optimum between 7.2 and 8.1.

*B. Natural (unsterilized) soils and water containing same*

In experiments with natural soils, a suitable quantity of air-dry Mukteswar soil was thoroughly mixed with two-fifths of its weight of tap water and amounts corresponding to 200 gm. of the dry soil were distributed in 250 c.c. bottles (bottom diameter 6.3 cm.). Some of the bottles were sterilized to act as controls and the water content then readjusted to equal roughly that of the unheated soil. The actual water content of all bottles was taken at the end of the experiment.

In the water experiments, thin layers of soil (50 gm.) were placed in rounded 500 c.c. flasks and filled with distilled water. Some were autoclaved to act as controls.

Anthrax spores were added in known numbers to the bottled soil and well mixed. Spores were similarly added to the water in the flasks. After various periods at 25°C. or 30°C., the soil was again mixed, the flasks were shaken and samples (4-8 gm. soil, 4 c.c. water) withdrawn for making dilutions,—for inoculation of guinea-pigs with the unsterilized medium and for plating with the sterilized control medium.

The results of several experiments are shown in Tables VI and VII. In experiments 13 and 14 (Table VI), soil alone was used; in experiments 15-17, horse manure (in form of 10 per cent watery extract), as well as lime and superphosphate to give final concentrations of 1.0, 0.1 and 0.02 per cent respectively, were added to the soil. In experiment 18, defibrinated ox blood 10 c.c. and water 100 c.c. were added to 200 gm. dry soil; after 6 days incubation, this medium became highly putrid and gave off abundant gas. From Tables VI and VII, it can be gathered that there is no evidence of growth taking place within 7-11 days in natural soil, even with the addition of substances which should produce a favourable effect. The same is true of unsterilized water standing over a thin layer of soil. On the other hand, under identical conditions, except that the media has been sterilized, growth took place within 5 or 6 days. It is important to note that only one out of 63 guinea-pigs died of intercurrent disease after inoculation with natural soil or water.

GROWTH OF *CL. CHAUVETI* IN SOIL

Tests were made at 25° or 30°C., usually the latter, using 100-200 gm. amounts of untreated Mukteswar soil, soil containing lime or ammonium sulphate to 0.1 per cent, soil containing lime to 0.1 per cent, superphosphate to 0.02 per cent and watery extract of horse manure to 1.0 per cent, (Table IV, legend). The water content was set at about 40 per cent. The number of viable organisms used for seeding the soil or the water in the

flasks varied in the different experiments from about 100-600 organisms per gm. of wet soil, except in one case where a very light seeding was used, viz. about 40 organisms per 100 gm. soil, and from 400 to 1000 organisms per c.c. of water. The number of organisms introduced into the soil or water was again checked immediately after seeding and was found to agree reasonably well with the expected number. In fact, in spite of the presumably large sampling error, the results throughout have been fairly concordant and should suffice to show all that was required, viz., whether growth was negligible or abundant. At intervals of from 5 to 49 days, soil or water samples were again examined. There were four separate experiments. It is unnecessary to give full details, for except in one instance, growth was slight or inappreciable, both in sterilized and in natural soil or water. The findings were consistent enough to indicate that, even with large seedings, growth, if occurring at all, is as a rule very restricted, usually not more than 10 times, occasionally as high as 100 times. There was an exceptional case where, in the presence of lime, superphosphate and horse manure extract, growth of *chauvoei* took place in one bottle from a very light seeding up to 2 million times within 15 days. From this experiment the impression was gained that growth was assisted by the presence of the lime, superphosphate and horse manure mixture but this could not be confirmed. Nor was there any real evidence that multiplication of *chauvoei* was enhanced by aerobes present in natural soil or water.

#### DISCUSSION

In considering the possibilities of pathogenic organisms multiplying in soil under natural conditions one has to take into account physical, chemical and biological factors. Soils vary in their composition, chemical reaction, temperature and water content, while various kinds of living organisms are present. Soil temperature and water content show great differences according to countries and seasons. The temperature is also to some extent dependant on moisture; in moist soil temperature rises are smaller than in dry soils, owing to the greater specific heat of moist soils. In the case of pathogenic organisms that might gain entrance, the surface layers would be mainly concerned. To deal with the temperature factor alone in illustration of the variability of conditions in soil at the surface the difference between daily maximum and minimum temperatures is large but the difference rapidly diminishes with increasing depth; at 6 in. depth at Rothamsted the daily fluctuations are practically inappreciable in winter but are marked in summer [Keen, 1926]. In England, according to Keen and Russell [1921], the mean temperature at 6 in. depth is about 20°C. in summer and 5°C. in winter. In subtropical countries such as India, the temperatures are, of course, much higher. Dravid [1940] has recently recorded that, at Poona, which has black soil and therefore absorbs greater amounts of heat through solar radiation than lighter-coloured soils, at 5 cm. depth the temperatures reach 30-32°C. in January, 32-42°C. in February-March, 47°C. in April and 31°C. in August. At 10 cm. depth the corresponding figures are: 26-28°C., 30-34°C., 40°C. and 62°C. During the summer in Poona, surface temperatures as high as 75°C. are occasionally recorded.



The observations set out in this paper do not support the view of those who believe that the anthrax bacillus may lead a saprophytic mode of existence. In a sense, therefore, it might be considered erroneous to speak of anthrax as a soil disease. It is admitted that in most countries soil temperature and moisture conditions might at times be suitable but there was no sign of growth in natural soil stored in the laboratory in bottles under favourable conditions and there seems to be no reason why the result should have been otherwise in the open. One may put it that the organism has no need to rely on saprophytism to maintain itself in the world, since in animals dying of anthrax the disease is septicaemic and hence there is a good chance of the surroundings becoming heavily contaminated. Considered from this point of view, the organism is a parasite as obligatory as, say, the tubercle bacillus both in hot and in temperate countries, but, at the same time, its chance of survival outside the host are high owing to the resistance of its spores.

We have seen little in the literature which might help one to visualize quantitatively the possible extent of ground contamination. Szpilman [1914] refers to an observation where one loopful of blood from a rabbit at the time of death produced some 4,000 anthrax colonies, or nearly 200 million bacteria in 100 gm. blood. We have taken the opportunity of making some further observations of the same nature. In the blood of guinea-pigs freshly dead of anthrax we have found the numbers of organisms to vary from about one to 25 millions in each drop. In the blood of 5 goats dying of anthrax in other experiments, the numbers of anthrax colonies (in millions) developing from 1.0 c.c. of blood were : 22, 50, 72, 260, 370. From the blood-stained nasal discharge of dead goats the number of colonies (in thousands) growing from 1.0 c.c. were : 250, 460, 520, 1000. In 4 goats the nasal discharge was not visibly blood-stained ; from 3 of these no anthrax colonies were obtained, from the fourth there were 18,000 per c.c. In a hill bull just dead of anthrax, the organisms in blood-stained nasal discharge numbered 800,000 per c.c. In another hill bull freshly died of anthrax, which we had the opportunity to examine, there were 2.5 million viable organisms per c.c. haemolysed blood taken from a peripheral vein and in the blood-stained nasal discharge there were 8,000 per c.c.

It cannot be said that the failure of the anthrax bacillus to grow in natural soil or water was entirely unexpected. The organism is known to be susceptible to the action of bacterial antagonists and these apparently suffice to restrain multiplication even when, by chance, temperature and other conditions are favourable. We have, in fact, isolated from Mukteswar soil two bacterial species which are definitely inhibitory to the anthrax bacillus.

In the case of *Cl. chauvæi*, the matter is not so straightforward, mainly owing to technical difficulties, while the literature only contains vague expressions of opinion which are of little value. From the knowledge which has been gained of mixed infections in the animal body in which anaerobes are present, we were prepared to find that in unsterilized soil or water aerobes might assist the multiplication of *chauvæi*. There has been no satisfactory evidence of this. It seems that, if the organism multiplies at all it is usually very restricted, even when extra organic matter is provided. If *chauvæi* grows apart from living tissues, it may be that it is in the body after death



and there is some evidence to show that this does happen. This, however, is a matter beyond the scope of this paper.

#### SUMMARY

1. The anthrax bacillus is capable of multiplying in heat-sterilized soils if the temperature is favourable, e.g. 25° or 30°C. and if they are sufficiently wet. A water content of about 20 per cent or over is suitable but the abundance of growth depends rather on the amount of 'free water' present. Within two weeks, increases up to 30,000 times were observed and with cessation of growth the organism sporulates.

2. Growth also takes place in soil enriched, individually or in mixture, with animal and plant manure, as well as with ammonium sulphate, lime and superphosphate. The addition of lime appeared to be particularly advantageous.

3. Tests with soils from various parts of India show that, in very acid soils, multiplication tends to be inhibited.

4. In natural (i.e., unsterilized) soil or water, placed under otherwise favourable conditions, there was no indication that the anthrax bacillus would grow, presumably owing to the antagonistic action of soil bacteria.

5. The evidence, therefore, strongly suggests that, in nature, the anthrax bacillus is mainly, if not entirely, an obligatory parasite. Its survival outside the body depends, firstly, on the resistance of its spores and, secondly, on the gross contamination of the surroundings, a point on which some quantitative data are given.

6. In the case of *Cl. chauvœi*, the experimental findings indicate that under favourable conditions growth of the organism is sterilized or natural soil, if occurring at all, is usually very restricted.

7. The literature on the subject of this paper is reviewed.

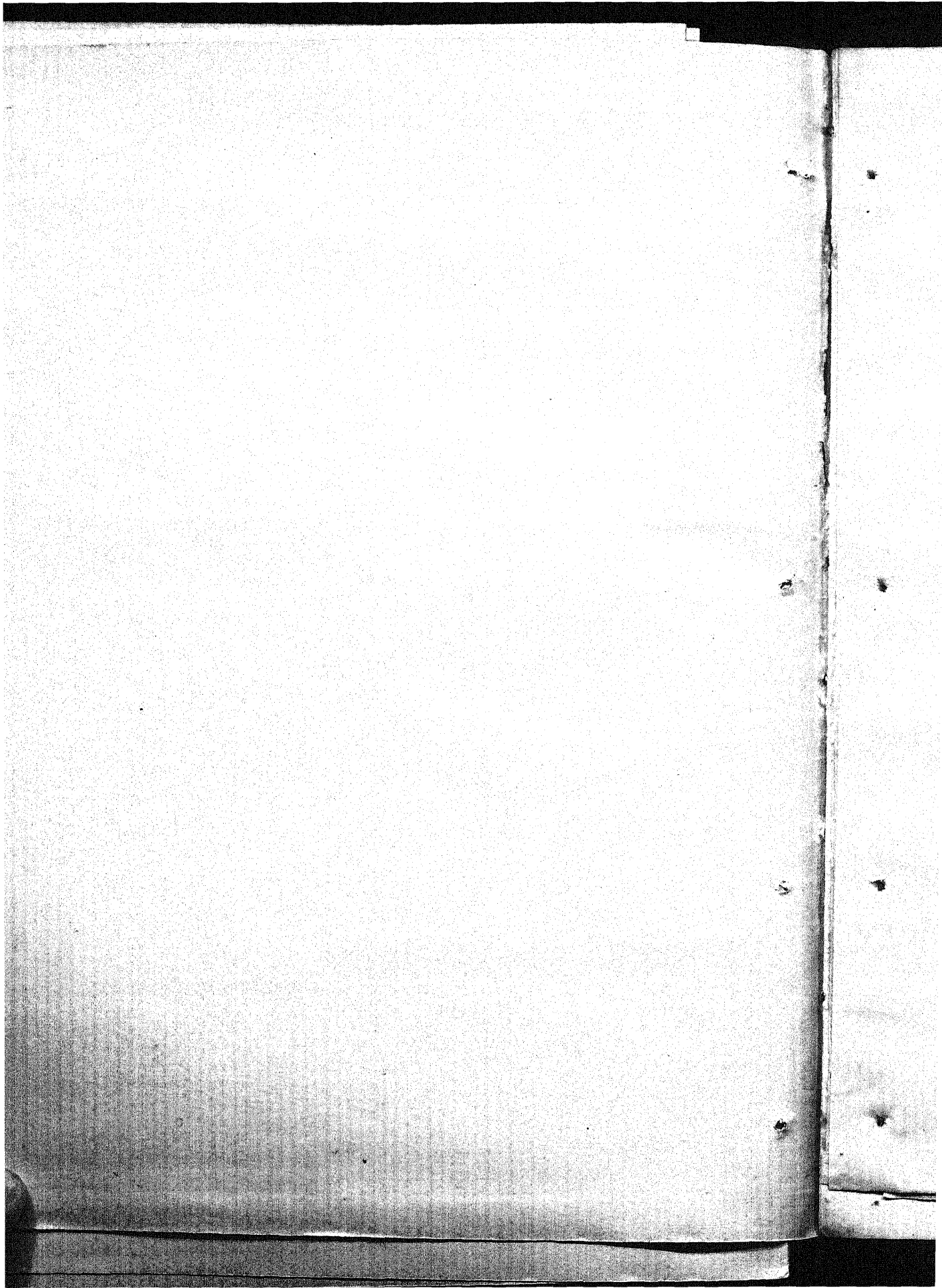
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Fine sand		Coarse sand		Nitro- gen	Car- bon	Organic matter	Ca. car- bonate	Lime status (per cent M. E. ex- changeable Ca)
(a)	(b)	(a)	(b)					
35.01 19.46 15.08 38.60	35.84 40.17 ... ...	39.21 60.90 37.60 ...	39.61 ... ... ...	... 0.108 0.085 0.098	1.16 1.20 1.04 1.10	2.01 2.07 1.70 1.90	0.065 0.0 0.0 37.4	5.43 10.25 0.05 7.30
1.11 3.49	... ...	7.78 38.07	... ...	0.040 0.049	0.83 0.50	1.43 0.86	9.8 7.1	48.00 37.60
12.83 2.25 14.17 11.85	... ... ... ...	28.88 59.94 28.88 58.85	... ... ... ...	0.038 0.038 0.082 0.097	0.52 0.43 0.86 1.00	0.90 0.74 1.43 1.72	0.0 1.05 0.4 0.6	5.75 21.40 2.50 1.25
9.7 16.7 19.3 8.6 7.4 15.4	... ... ... ... ... ...	17.7 54.6 45.5 4.1 2.9 59.9	... ... ... ... ... ...	0.043 0.031 0.060 0.005 0.064 0.045	... ... ... ... ... ...	0.890 0.726 0.549 0.901 *0.865 0.867	... ... ... ... ... ...	32.4 9.2 6.8 30.0 33.6 5.2

\*thod.



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TABLE VII

*Attempted growth of anthrax in water containing natural soil at 30°C.*

Number of experiment . . .	18	19	20	18	19	20
	Natural water			Sterilized water (control)		
Estimated number of anthrax bacteria added per c.c.	240	170	78	240	170	78
Plated or injected—						
Immediate . . . . .	(b) S, S	(b) S, S	(b) S, S	(b) 4	(b) 2·8	(b) 1·3
After 5 days . . . . .	...	(c) S	(c) S	...	(b) 20	(b) 205
" 6 " . . . . .	(c) S	...	...	(b) 26	...	...
" 7 " . . . . .	(c) S (Undil.) D5	...	(c) S	(c) S (Undil.) D2	...	...
" 11 " . . . . .	...	(c) S	...	...	...	...

(a) Dilution of water at rate of 4·0 c.c. to 20 c.c. saline; (b) and (c) are tenfold dilutions from (a).  
 The number of colonies under 'immediate plating, has been estimated for dilution (b) from the number of anthrax bacteria added to the water.  
 Other legend as for Table VI.

# THE VITAMIN A CONTENT OF COW'S BUTTER AND GHEE AND OF BUFFALO GHEE

BY

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(Received for publication on 21 July 1941)

BUTTER and ghee have been analysed from time to time by various workers for their vitamin A activity [Morgan and Pritchard, 1935, 1937; Baumann *et al.*, 1934; Baumann and Steenbock, 1933; Gillam *et al.*, 1936; Bacharach, 1930; Grewal, 1933; Grewal and Kochar, 1938; De and Majumdar, 1938]. The values in most cases have been recorded in terms of Carr-Price or Blue values, instead of the easily understood International Units. For comparative work, this has introduced difficulties as there are no recognized conversion factors for the expression of Blue values in terms of International Units. Apart from the above reports, little information was available on the vitamin A contents of butter and ghee prepared from the same source. Since butter and ghee, particularly the latter, are important constituents of Indian diet, it was thought desirable to undertake a systematic survey of this question and to present the results in International Units.

Ten samples of fresh butter (cow's\*), were therefore, obtained from the Government Dairy Farm, Wellington, where it was specially prepared on successive days without the addition of salt or colouring matter. Ghee was prepared in the laboratory from these samples in open porcelain dishes over a naked flame. The spectrophotometric method of assaying vitamin A and carotene was adopted. The principle and details of the method are similar to those described by De [1937]. The band at 328 m $\mu$  was taken as characteristic of vitamin A and that at 463 m $\mu$  of carotene. The bases of calculation were 1,600 for vitamin A and 1,900 for carotene. In this connection, 18 samples of buffalo ghee (sent by the Agricultural Marketing Adviser to the Government of India) were also analysed as it was considered important to know the difference in the vitamin A activities of cow and buffalo ghee. The results are recorded in Tables I and II. In tabulating the data, one microgram of vitamin A was taken to be equivalent to 1.56 I.U. and one microgram of carotene equivalent to 1.66 I. U. [Morgan *et al.*, 1935].

\*The cows from which the butter was obtained were mostly cross-bred animals.



# 330 *Vitamin A Content of Cow's Butter and Ghee and Buffalo Ghee*

TABLE I

*Vitamin A and carotene content of cow's butter and of ghee prepared from the same material*

Sample	Material	Moisture content	Vitamin A per gm. of fat I.U.	Carotene per gm. of fat I.U.
1	Butter	16.0	17.5	10.8
	Ghee	..	14.7	9.3
2	Butter	13.0	15.6	9.0
	Ghee	..	13.7	8.0
3	Butter	19.0	18.1	11.5
	Ghee	..	14.7	9.1
4	Butter	17.0	15.3	8.8
	Ghee	..	13.0	7.4
5	Butter	19.0	20.4	8.7
	Ghee	..	16.2	6.6
6	Butter	15.4	19.7	8.7
	Ghee	..	17.0	7.3
7	Butter	15.2	15.0	6.3
	Ghee	..	12.2	5.0
8	Butter	17.1	20.0	8.8
	Ghee	..	15.4	6.6
9	Butter	18.1	19.0	8.5
	Ghee	..	15.4	6.6
10	Butter	16.2	16.2	3.3
	Ghee	..	9.0	1.8

TABLE II

*Vitamin A content of buffalo ghee*

Sample Nos.	Vitamin A per gm. of fat
1	0.98
2	1.98
3	0.73
4	2.43
5	2.93
6	1.21
7	1.98
8	1.21
9	1.45
10	0.97
11	1.45
12	1.45
13	3.40
14	2.43
15	2.90
16	3.40
17	1.98
18	1.47

The average vitamin A value of the cow butter samples was found to be 17.7 I.U. per gm. of fat and the carotene content 8.4 I.U. The vitamin A content and the carotene values of ghee samples prepared from the above butter were on an average 14.1 I.U. and 6.8 I.U. per gm. respectively; while, with buffalo ghee the average vitamin A value observed was 1.9 I.U. per gm. and traces only of carotene were found to be present. These are within the range of those reported by some other workers.

Next, in continuation of previous work [De and Majumdar 1938], it was thought worthwhile to study how far the period of heating and the maximum temperature reached in the course of the preparation of the ghee samples were responsible for the destruction of the vitamin A originally present. The results tabulated in Table III show that the loss of vitamin A during the preparation of ghee was on an average 17.4 per cent and depended on the temperature and the period of heating.

TABLE III

*Effect of temperature and the period of heating on the vitamin A content of ghee*

Sample Nos.	Period of heating (in minutes)	Maximum temperature reached (in °C.)	Loss of vitamin A per cent
1	30	125	16.0
3	"	138	19.0
7	"	135	19.0
9	"	138	19.0
2	20	140	12.0
4	"	155	15.0
6	25	130	13.5
5	35	140	20.6
8	45	150	22.6
10	90	156	44.0

Loss during the preparation of ghee (average of nine samples excluding sample No. 10) . . . . . 17.4 per cent.

## SUMMARY

Ten samples of fresh cow's butter and ten samples of ghee prepared from this butter were analysed for their vitamin A and carotene contents by the spectrophotometric method. The vitamin A value of the cow's butter was found to be from 15 to 20 I.U. per gm. of fat and the carotene content from 3 to 12 I.U., the total vitamin A potency being about 11,800 I.U. per pound of butter. The vitamin A content and the carotene values of ghee prepared from the above butter ranged from 10 to 17 I.U. and from 2.0 to 9.0 I.U. per gm. respectively. The moisture content of the butter samples varied from 13 to 20 per cent. The loss of vitamin A activity during the preparation of ghee was on an average 17.4 per cent and depended on the temperature and the period of heating. Eighteen samples of buffalo ghee were also analysed. The vitamin A values ranged from 1 to 3.5 I. U. per gm. Traces only of carotene were present.

## ACKNOWLEDGEMENTS

The experimental work presented in this paper was completed at the Nutrition Research Laboratories, Coonoor, during the tenure of a Parlakimedi Research Scholarship. Acknowledgement is made to the Indian Research Fund Association and the Director, Nutrition Research Laboratories, Coonoor, for the facilities afforded to the author in his work. Thanks are also due to Dr K. C. Sen, D.Sc., Officer-in-charge, Animal Nutrition Section, Izatnagar for his encouragement.

## Note

After the present communication was sent to the press, an article on the Vitamin A content of ghee by M. C. Muthanna and P. K. Seshan (*Ind. Med. Gaz.* 1941, **76**, 487) has appeared. The average values of the vitamin A content of Bengal and Sind ghee, as found by these authors, agree fairly well with the results reported in this paper.

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ON THE NATURE OF THE URINOGENITAL PAPILLA OF  
*CLARIAS BATRACHUS* (LINN.) AND *HETEROPNEUSTES*  
*FOSSILIS* (BLOCH)

BY

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(With two text-figures)

IN a recent article by the authors [Mookerjee, Mazumdar and Das Gupta, 1940] attention was directed to the differences between the external urinogenital structures in the males and females of the species. In the present article they propose to deal with similar structures in two other catfishes popularly known as 'Magur' and 'Singi' which are of great economic importance in Bengal.

The presence of urinogenital papillae or the so-called pseudo-copulatory organs has been recorded in a number of siluroid and other fishes [Dean, 1923]. More recently Hora and Law [1941, 1 and 1941, 2] described such structures in certain species of *Mystus* (= *Macrones*) and *Batasio* (Family: Bagridae), and in *Gagata* (Family: Sisoridae), but so far as the authors are aware the urinogenital papillae of 'Magur' and 'Singi' which belong to the genera *Clarias* (Family: Clariidae) and *Heteropneustes* (= *Saccobranthus*) (Family: Heteropneustidae) respectively have not yet been described.

In these two fishes the anus and the urinogenital openings, although distinct from each other, are placed close together; the anus being in front of the urinogenital opening. The anal opening in both the sexes is an ovoid aperture, guarded by the anal sphincter. The ureters open into the bladder. The urethra and the genital duct in the females open separately while in the males the urethra and the genital ducts are confluent. The urinogenital papilla is a free, muscular, sac-like structure, which arises from the midventral surface of the body shortly behind the anal opening; it is visible to the naked eye and can easily be raised with a needle. In *C. batrachus* the comparatively stouter papilla is placed much nearer the anus than it is in *H. fossilis*. In the females the papilla is more flush with the body surface than it is in the males, particularly in *H. fossilis*.

In *C. batrachus* the urinogenital papilla of the male is elongated and narrower than that of the female; its free end is pointed in the male and relatively much broader in the female (Fig. 1).

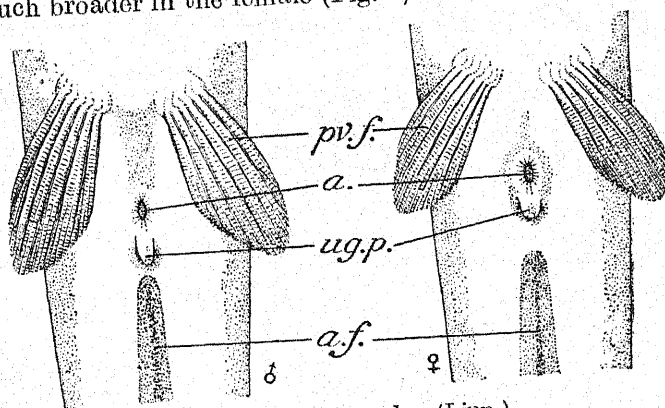


Fig. 1. *Clarias batrachus* (Linn.)

In *H. fossilis*, the urinogenital papillae of both sexes are more or less like those of *C. batrachus* except that in the former, the papillae are comparatively narrower and more pointed (Fig. 2). There is a median cleft on the ventral surface of the female urinogenital papilla representing the urinogenital opening, while in the male this opening lies at the tip of the papilla.

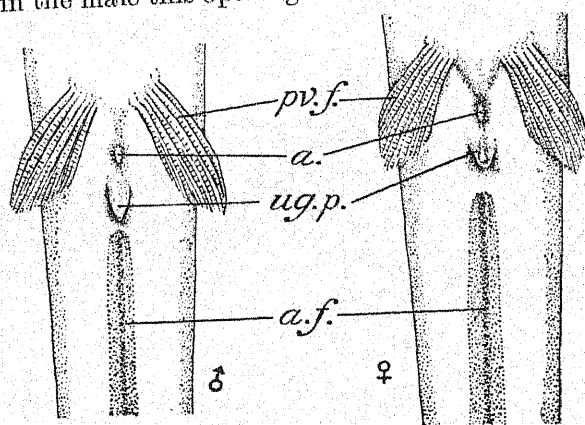


Fig. 2. *Heteropneustes fossilis* (Bloch)

During the spawning season, the papilla of the females becomes swollen<sup>1</sup> and the cleft becomes vascular. Since these fishes are highly prized as diet for invalids in the convalescent state and since there is also a belief that the male specimens are superior to the females in respect of their taste and food value, the shape of the papilla should prove useful for identifying the sexes. Some means to determine the males and females will also prove helpful for the culture of these important edible fishes.

<sup>1</sup> The swelling is very marked in specimens fixed in 4 per cent Formaldehyde.

## ACKNOWLEDGEMENT

## REFERENCES

- ### EXPLANATION OF TEXT-FIGURES

1. Urinogenital papilla in the male and female of *Clarias batrachus* (Linn.).  $\times 1 \frac{1}{3}$   
*a.* Anus; *a. f.* Anal fin; *pv. f.* Pelvic fin; *ug. p.* Urinogenital papilla.
2. Urinogenital papilla in the male and female of *Heteropneustes fossilis* (Bloch).  $\times 1 \frac{1}{3}$   
*a.* Anus; *a. f.* Anal fin; *pv. f.* Pelvic fin; *ug. p.* Urinogenital papilla.

## SELECTED ARTICLES

### EQUINE ENCEPHALOMYELITIS IN INDIA

BY

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(With Plates XXII—XXVI)

(Reprinted from The Journal of The Royal Army Veterinary Corps, London, Vol. II, Nos. 2 and 3.)

#### I.—INTRODUCTION

“THE subject of neurotropic viruses is, today, one of the actively advancing wavelets on the tide of medical science. Of late, many new neurotropic viruses affecting man and animals have been isolated and it is quite clear that the discovery of others awaits only the application of the experimental method to cases of hitherto obscure nervous illness.” These words of Hurst epitomise the reorientation of thought which has revolutionized, during the last two decades, earlier opinions on the nature of certain diseases of the nervous system.

The term ‘mad staggers’, one whose origin is lost in antiquity, has, until recent years, been employed to designate those obscure illnesses in which cerebral and nervous symptoms predominate. For centuries, every conceivable theory has been advanced to account for that group of diseases of which equine encephalomyelitis is an example. Until recent years, the protagonists of the metabolic error theory have held sway, on what grounds it is not clear. But in a wide toxicological field ranging from protein intoxication to plant toxæmias, the term ‘forage poisoning’ has attracted its own band of adherents.

This practice of explaining the obscure by the still more obscure was finally demolished by the epoch-making work of Zwiek and his co-workers [1925], who proved conclusively that one form of equine encephalomyelitis (Borna disease) is specifically caused by a neurotropic virus.

These findings were anticipated by Landsteiner and Popper [1909], who, investigating an analogous disease of the human subject—anterior poliomyelitis—drew attention to a new and complex syndrome of obscure nervous illness which, they showed, is due to the invasion of the central nervous system by a virus with a specificity for these tissues.

These discoveries opened up an immense field of investigation into the etiology—and pathology—of obscure nervous diseases of animals, and within a short space of time Nicolau and Galloway [1927] had shown that a variety of domesticated animals is susceptible to a specific encephalomyelitis caused by the virus of Borna disease, and only a year or two later, Pool, Gordon, Brownlee and Wilson conclusively proved the identity of “louping ill”: while McLeod indicated the natural method of transmission by the common tick (*Ixodes ricinus*).



Incidentally, about this time, American Veterinary opinion was forsaking the time-honoured term "forage poisoning" in horses, in favour of the term "equine encephalomyelitis", it having been proved by Mayer, Howitt and others [1931] that the common meningo-encephalitis of horses is in reality a specific encephalomyelitis associated with a neurotropic virus. Somewhat later, it was shown that two types of the disease exist in North America, the Eastern and Western, each associated with an immunologically distinct virus.

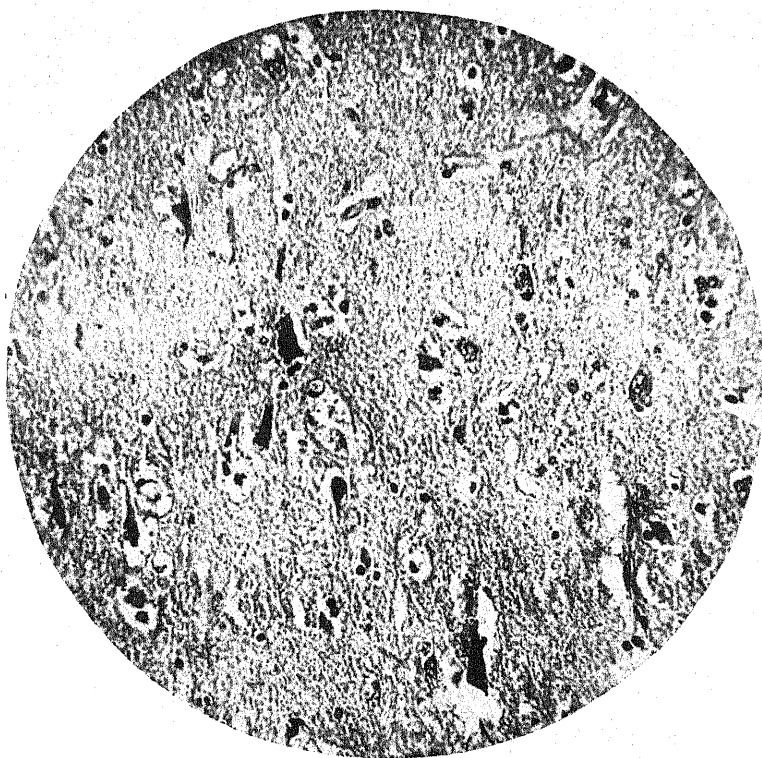
In India, Mosley, Heane and Shirlaw [1934] proved the existence of equine-encephalomyelitis and adduced some evidence to show that the disease bears a distinct relationship to the form of equine encephalomyelitis described by Moussu and Marchand in France [1924], a disease which, they said, was probably imported from America during the World War. Mosley, Heane and Shirlaw [1934] expressed the opinion, based on pathological findings and experimental transmission in two horses, that the disease, in India, is probably due to a neurotropic virus.

This revolutionary concept of the etiology of obscure nervous diseases of animals was speedily succeeded by difficulties in interpretation of the syndrome and pathological feature. Following upon the acceptance of a virus etiology, more attention came to be paid to pathological differences, while variations in the type of virus readily became apparent.

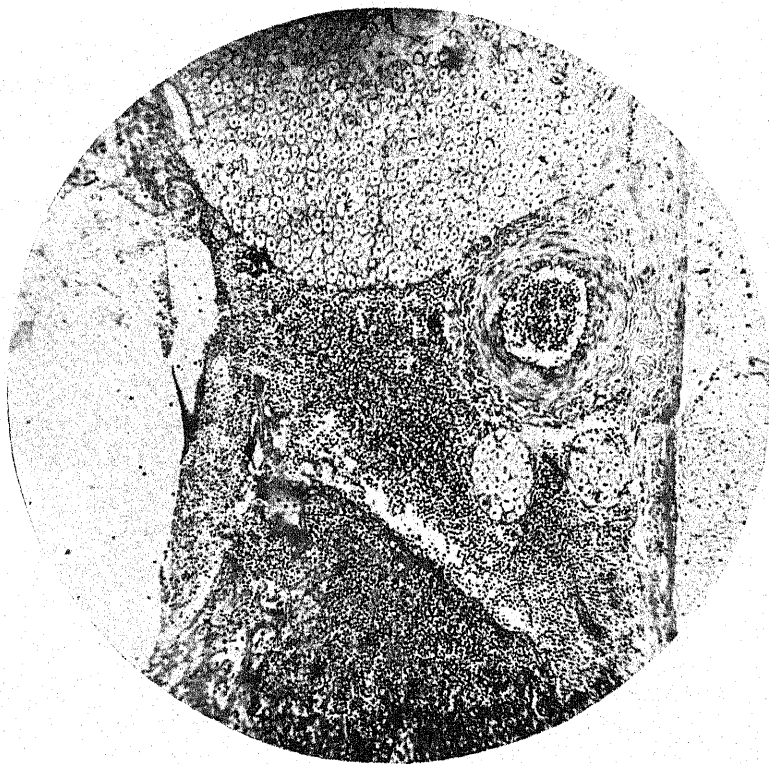
Moussu and Marchand claimed that the French form of the disease possessed no radical difference from Borna disease, an opinion which Nicolau and Galloway promptly refuted on the grounds that, in Borna disease, the lesions are essentially confined to the central nervous system and intranuclear inclusions, considered specific of Borna disease, are absent in the disease described by Moussu and Marchand.

Accepting this opinion, the type of equine encephalomyelitis occurring in America would appear to be an intermediary form. As in Borna disease, lesions of the internal organs are either absent or inconspicuous. Intranuclear inclusions are considered not to be a specific diagnostic feature of the American form, although Hurst, in a study of the Western type describes the presence of intranuclear inclusions in the nerve cells of the tissues which he examined.

These differences have pointed to the probability that several types of neurotropic virus exist, each tending to a general and similar syndrome, influenced by common epizootic factors. There has been, moreover, a recent tendency to consider that all neurotropic viruses are not essentially parasitic on nerve cells. Certain of them, probably, while evincing a predilection for nerve cells, are not entirely selective, and may invade the tissues of the internal organs with resultant lesions therein. In other words, certain neurotropic viruses possess organotropic affinities. More recently, it has been shown that the viruses of pox and measles and vesicular stomatitis, previously considered as dermatropic and organotropic, possess considerable neurotropic affinities. It appears probable that certain of the equine encephalomyelitis viruses are essentially neurotropic with organotropic affinities, and this probably accounts for the variations in syndrome and pathological expression in a disease which is fundamentally basic in type.



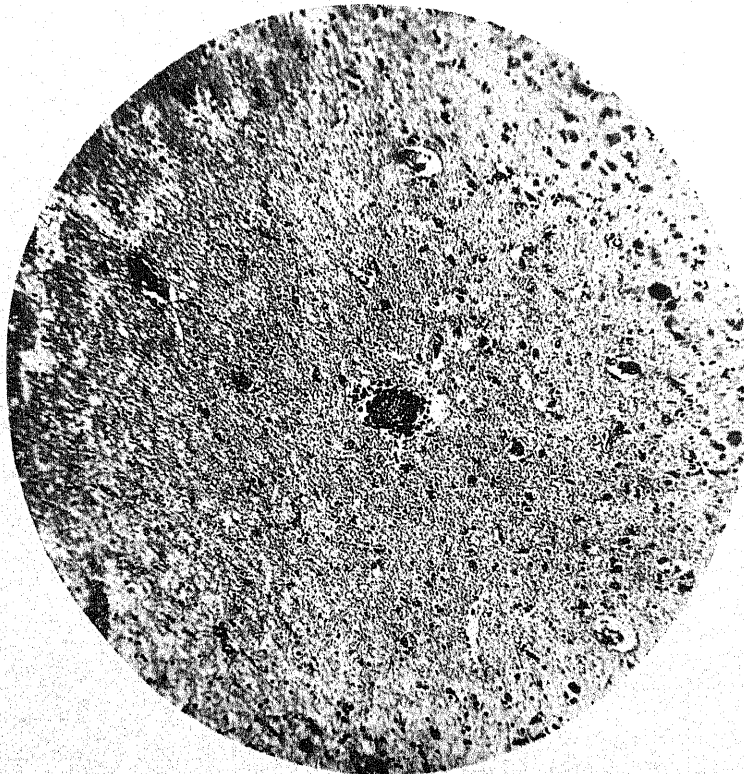
SECTION OF CEREBRAL (PARIETAL) CORTEX  
Intense neuronophagia



SECTION OF SPINAL CORD AFFECTED WITH SUBPIAL HAEMORRHAGE  
Note the separation of and pressure upon nerve roots, and the  
markedly sclerotic appearance of an arterial branch



SECTION OF PARIETAL CORTEX (FRONTAL LOBE)  
Marked perivascular "cuffing," neuronophagia and diffuse monocyctosis



SECTION OF CEREBRAL (PARIETAL) CORTEX  
Perivascular infiltration marked, with diffuse infiltration of  
monocytes and total disappearance of nerve cells



Hurst has attempted a classification of neurotropic viruses according to the distribution of the lesions encountered on histological examination of the brain, a feat which presupposes that one is dealing with a pure single type virus and one somewhat complicated by the fact that small laboratory animals are in themselves liable to a specific encephalomyelitis, which may flare up on the depressant action induced by the inoculation of a neurotropic virus isolated from a different animal species. Hurst sums up these difficulties in expression, from the point of view of etiological, pathological and clinical considerations, in one pregnant sentence: "The various forms of virus encephalomyelitis bear no closer relation, one to another, than does smallpox to measles."

When we remember the intense concentration of highly skilled workers in those countries afflicted with the disease and the controversies that have arisen, the difficulties of the isolated worker in India, on similar problems, may readily be realised.

## II.—HISTORY OF THE DISEASE AND ITS DISTRIBUTION IN INDIA

Equine encephalomyelitis is not a disease new to India. Ancient Hindu writings refer to a disease which Lécainche identifies as a specific meningo-encephalitis. In a Persian translation made by order of the Emperor Shah Jehan of a Sanskrit compilation 'by a society of learned pundits' called 'Saloter', reference is made to a 'dreadful malady' of horses which is recognizable as "mad staggers" and in which the symptoms bear an undoubted resemblance to present day equine encephalomyelitis. A variant of this condition—in the same writing—is attributed to the horse's being seized by 'Wind' in the hind quarters, and the ensuing description conforms to the more modern descriptions of Kumri. Moorcroft, one of the earliest veterinary pioneers in India, regarded Kumri as a cerebral affection and demonstrated an excess of cerebro-spinal fluid which could be 'drained off the dead subject by opening the occipito-atloid articulation'. (Smith—"History of Veterinary Literature".)

Current veterinary opinion in India considers that equine encephalomyelitis and Kumri are distinct and separate disease entities. The term "encephalomyelitis" is used at the moment, therefore, to denote that symptom-complex which the writer first observed in the recorded outbreak at Multan [1933] and which corresponds in symptomatology with that recorded by workers in other countries where the disease is enzootic. These symptoms are referable, chiefly, to involvement of the higher nerve centres, although no case is ever encountered in which the higher and lower (spinal cord) centres are not simultaneously affected. It is important to observe that Kumri is considered a sporadic disease with subacute symptoms and equine encephalomyelitis an epizootic disease with acute or peracute symptoms.

It is a surprising fact that equine encephalomyelitis only sprang into prominence in 1933, since when it has assumed great importance and occasioned much thought among veterinary workers in India. A study of existing records shows, however, that the increase of the disease may be more apparent than real and that many outbreaks have passed unnoticed or been ascribed to other causes.



Poyser writing on Kumri [1885] states: "The question of causation attracted more than ordinary attention whenever it (Kumri) assumed an epizootic character and was unusually fatal. Further opportunity (to study the disease?) was not met with until August, September and October 1884 when so-called Kumri appeared in Meerut in an *epizootic form* among the horses of the 8th Hussars and among private horses. Such cases occurring simultaneously at Saharanpur have been called Kumri, at Ambala and Muttra paralysis, and in Morar, *Brain disease* or paralysis. Kettlewell believes that some cases are complicated with cerebro-spinal meningitis and it is not unlikely that this complex phase may obtain, as he says, in some and possibly in the worst cases. It is to be distinctly understood that the recent equine *epizootic* in Meerut is no new disease: it is simply a very old one with a new name and the same with which the writer had to contend in Meerut twelve or thirteen years ago, there and then registered as Kumri or paralysis."

Lingard [1896] describes an outbreak of equine paralysis which he investigated at Karnal (Punjab) and comments on peculiar 'acute cases'. It is interesting to note that Lingard ascribed this disease to forage poisoning.

Similar outbreaks occurred at Tinnevely (Madras) in 1914-15, Secunderabad (Deccan) 1916 and Saharanpur 1923. Among four hundred and twenty five horses at Secunderabad, twenty-two contracted paralysis, fifteen of which had to be destroyed, one dying of the disease. At the same time, four chargers of the 2/7th Hampshire Regiment became affected at a fresh centre in Secunderabad and three died. It appears obvious, therefore, that an acute cerebral form of the disease (equine encephalomyelitis) was not unusual, in epizootic form, in certain areas in India where Kumri was rife.

The observations of these workers constitute the earliest efforts in organised veterinary work in India. The subsequent history of the disease may be gleaned from the Annual Administration Reports of the Army Veterinary Service in India. In 1921-22, sixty horses were affected with "disease of the nervous system", eight died and twenty-five were destroyed. Horses, mules, buffaloes and camels are also noted to have been affected with cerebro-spinal meningitis, encephalitis, congestion of the brain, epilepsy, general paralysis, paraplegia, vertigo, chorea and hemiplegia, and it appears obvious that a considerable proportion of these cases, at least, were either Kumri or encephalomyelitis. Succeeding reports indicate the comparative and fluctuating frequency of these affections.

Outbreaks of equine paraplegia were recorded at the Army Remount Depots at Sargodha, 1923, at the same time as an outbreak at Saharanpur, but it was not until the year 1926-27 that the army reports make mention of a serious outbreak of 'forage poisoning' at Jhansi (U. P.) in the horses of the 16th Light Cavalry. The outbreak commenced in September, 1926 and within a few days, sixty-one animals were affected, of which twenty-nine died. In addition to this outbreak, eight cases of a similar nature occurred at Mona Remount Depot during October, five cases dying and one recovering. The disease reappeared at Mona in February 1927, six horses being affected with five deaths. These cases were attributed to sorghum poisoning, but the clinical histories suggest equine encephalomyelitis.

The following year at Jhansi (August 1927) a serious outbreak of 'tympantitic colic' broke out in the horses of the regiment affected during the preceding year and the 31st Mule Transport Company. Within nine days, sixty cases occurred, with ten deaths. This outbreak was ascribed to forage poisoning and guinea grass was considered a possible etiological factor. It appears probable, from such meagre records as obtain, that the disease was a recrudescence of the disease noted during the previous year.

During January, 1930, fourteen cases of 'poisoning' occurred among the horses of the 22nd Field Battery R. A., Lucknow, of which one died, two developed paraplegia and were destroyed, and eleven recovered. This outbreak, undoubtedly one of equine encephalomyelitis, was considered to be due to soaked gram fed to the horses.

In the winter of 1930, Simpson investigated an outbreak of disease in army horses at Rawalpindi. The symptoms were those commonly associated with equine encephalomyelitis and the cause was ascribed to ratti seed poisoning. At about the same time, fourteen cases of a similar disease occurred at the Remount Depot, Saharanpur. This outbreak was diagnosed as paraplegia and the fodder incriminated as the source of origin.

The year 1933 saw the severest and most extensive outbreak of paraplegia which has affected a cavalry regiment and a thorough investigation showed the disease to be a true encephalomyelitis which does not differ in any vital respect from the same disease which has given rise to a great wealth of literature in other countries in recent years.

A second severe outbreak of equine encephalomyelitis occurred in the same regiment during the winter of 1935-36, but no major epizootic has since occurred. Minor outbreaks, in which two or three horses may be affected, have, however, been frequent during the last few years, while sporadic cases have, undoubtedly, gained in frequency.

Equine encephalomyelitis appears firmly rooted in certain areas in India which have now come to be looked upon as enzootic areas and there appears no doubt that today the disease is on the increase.

### III.—COMMENTARY ON THE SEQUELAE OF THE MULTAN OUTBREAK

The outbreak of equine encephalomyelitis in the horses of the 13th Lancers at Multan has been described in some detail [Mosley, Heane and Shirlaw, 1934]. It commenced in November 1933 and terminated in March 1934, as spontaneously as it had started, a feature of all outbreaks of equine encephalomyelitis. Seventy horses had been affected, twenty-one of which had either died or been destroyed.

One is left in doubt, however, as to whether these figures represent the total incidence of the disease and whether many horses did not pass through a fleeting phase which escaped notice. In human poliomyelitis 'it is only occasional cases, attributed by some at less than one per cent of the total infected, which develop paralysis and are diagnosed as such'. (Hurst.)

In equine encephalomyelitis, which, as will be shown later is also a poliо-encephalomyelitis, it is not irrational to believe that a similar phase in infection occurs. This belief is borne out by the observation that, when routine

temperature recording of all incontact horses was adopted, towards the end of the outbreak, ten horses suddenly developed pyrexia. Five of these—the last five cases in the regiment—developed equine encephalomyelitis on the abatement of fever, a few days later. It is considered probable that if temperatures had been recorded at the beginning of the outbreak, more cases of this nature would have been discovered. In this, and in later outbreaks, a similar phase has been noted and one is led to believe that in acute encephalomyelitis, the disease is ushered in by a transient pyrexia and that all infected horses do not proceed to a manifestation of symptoms. In fact the wide variation in syndrome seen in the Multan outbreak, is capable of explanation on the assumption that, once infected, cases vary in symptomatology between no-reaction, mild, and severe reaction, as in human poliomyelitis.

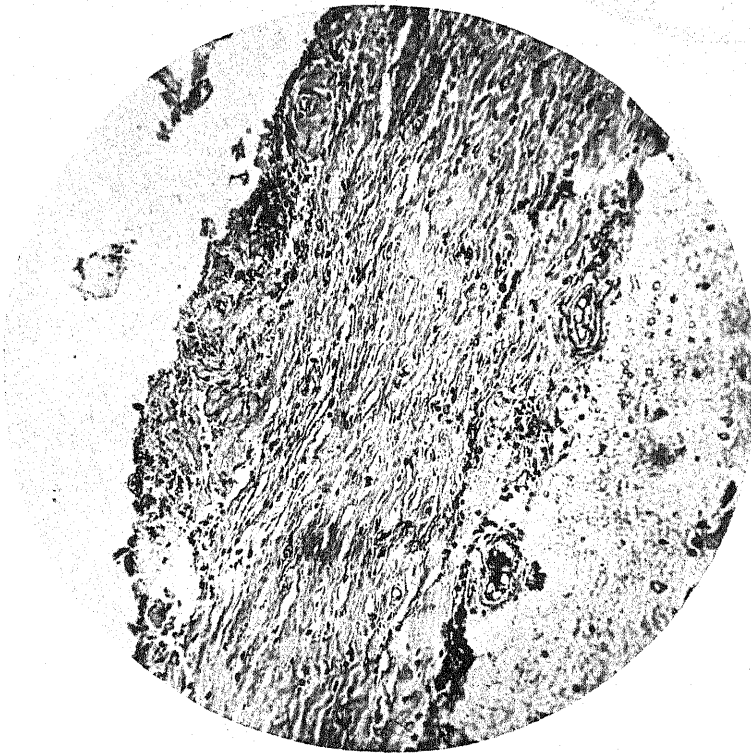
On the cessation of the outbreak, the regiment proceeded to Jullundur. One year later, three cases, horses Nos. 62, 233 and 527, were noticed 'weak in the loins'. 233 was a pyrexia case at Multan and 62 and 527 had mild paraplegia at Khanewal in November, 1933. All three had gone down under their riders and been made to rise with difficulty, shivering and trembling and scarcely able to return to stables. Within a few days, a typical paraplegia developed with marked incoordination of movement. The tail was held high and laterally retracted and constant dribbling of urine (vesical paralysis) was noted. The posterior quarters were insensitive to forceful pin pricks and phases of slowly abating hyperæsthesia could be readily invoked. The ocular reflexes were poor and spasmodic twitching of groups of the face, ear and cervical muscles was evident. The nerve tissues of these animals were subsequently utilised for transmission experiments in small animals. With this minor recrudescence, the disease in the regiment was considered to have cleared up (May, 1935).

In January 1936, however, the disease suddenly flared up again. For a few days prior to onset, there had been some rain and a sudden fall in temperature. The outbreak lasted for about ten weeks, during which time minor waves of the infection swept over the horses, and at the beginning of the warm weather, the disease cleared up spontaneously. Eighteen cases had occurred, three had been destroyed and one died.

A peculiarity of this outbreak—the second to be critically observed in all its features—was a decided tendency for independent sporadic cases, with no apparent relationship or contiguity, to occur at irregular intervals of a few days. The disease was of a mild nature in comparison with the Multan outbreak. Only three cases developed marked cerebral symptoms. The remainder might easily have been termed enzootic spinal paraplegia with a tendency to cerebral involvement.

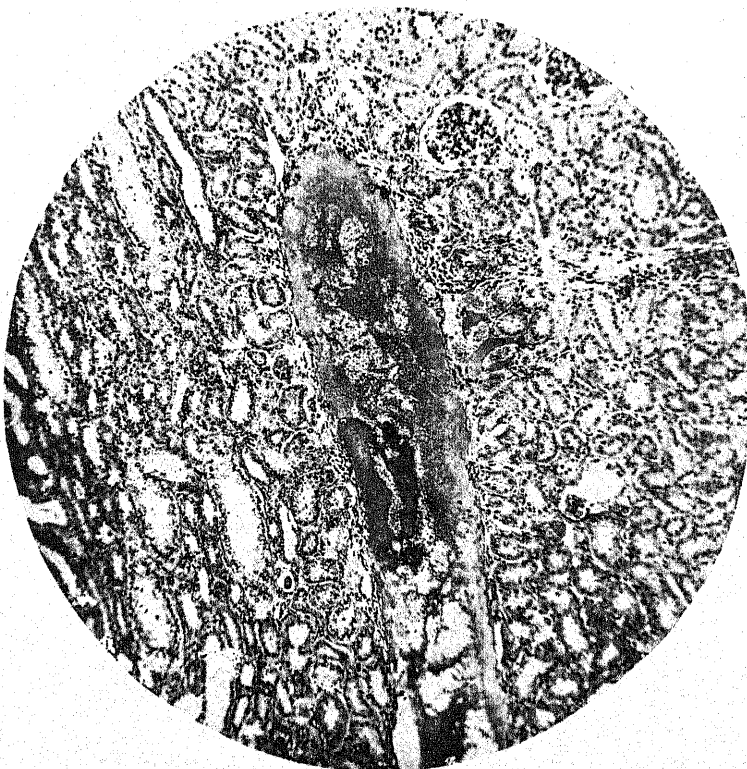
It was, however, a definite outbreak of equine encephalomyelitis. Three cases showed undoubted clinical symptoms and *all* cases the specific lesions of the disease after death. It was further observed, during the course of this outbreak, that a considerable number of cases developed pyrexia, followed, in one or two cases, by the onset of symptoms with the remission of fever. In brief, all the epizootic factors and clinical details observed in the Multan outbreak were further substantiated as being illustrative of a field outbreak of equine encephalomyelitis.





CHRONIC FIBROUS LEPTO-MENINGITIS

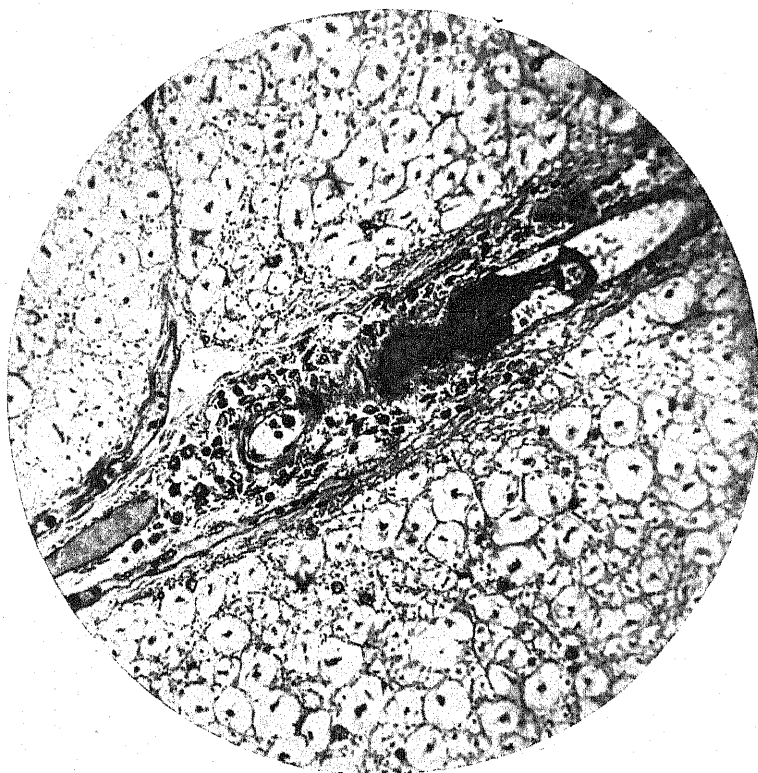
An unusual and somewhat rare lesion in encephalomyelitis



SECTION OF KIDNEY

Note the considerable area of haemorrhage, the slight interstitial hyperplasia, and the comparative normality of the tubular epithelium





SMALL ARTERY IN WHITE SUBSTANCE OF CORD

Marked perivascular infiltration, over-distension and early rupture  
of the homonymous vein

These, however, are the most important points of comment:—no previous case of equine encephalomyelitis, so far as one is aware, had ever occurred in Jullundur. The horses of the 13th Lancers on their way from Multan to Jullundur had been rigorously quarantined *en route*. There was, therefore, no conceivable possibility that the three cases noted early in 1935 and also the main outbreak of January (1936) were in any way connected with a fresh infection. The only possibility that remains, therefore, is that 'carriers' existed in the regiment.

The study of the subsequent history of the horses of the 13th Lancers at Jullundur afforded two valuable items of information. The epizootology and clinical syndrome noted during the course of this outbreak of equine encephalomyelitis corresponded exactly with that observed at Multan and one is therefore justified in concluding that they are specific of the disease. 'The question of carriers'—strongly stressed by Heane on *prima facie* evidence at Multan—'cannot be ignored' and there seems no other feasible explanation which can be accepted, or for which there are any grounds, to account for the second outbreak in this regiment.

#### IV.—THE CLINOLOGY DURING (a) THE COURSE OF THE OUTBREAKS AND (b) SPORADIC CASES

In the earlier outbreaks, so mystifying and variable was the syndrome, so peculiar the epizootic and pre-disposing factors, so negative the post-mortem findings—apart from a fairly constant gastroenteritis—that it is not surprising that the earlier observers were inclined to ascribe the disease to unidentifiable plant and fodder poisoning. The tendency of certain Indian fodders and grasses (*e.g.* sorghum) to be toxicogenic (cyanogenetic glucosides) at about the time when the disease made its appearance, lent a very strong, if circumstantial, support to the toxicological theory. The symptoms were, therefore, in common with most obscure plant poisonings, described as indefinite, with a tendency to cerebral manifestation.

During the years 1923-33, when equine encephalomyelitis was first diagnosed, the disease was described as paraplegia or simply paralysis in the milder cases—many of which recovered—and cerebral meningitis in the severe cases which were usually fatal. Several of the mild paraplegia cases were noted to progress, or relapse, to the acute cerebral phase. Generally, the majority of cases were paraplegic, of varying degree, and while some apparently made an uneventful recovery, others, progressive cases, had to be destroyed as hopeless. The ratio of recoveries to hopeless cases was variable in each outbreak.

In the outbreak at Saharanpur (1923 September–December), fourteen horses were affected, only one showed acute cerebral symptoms at the onset, five progressed from a mild paraplegia to the cerebral type within two to three days and seven were destroyed as hopeless cases, all of them showing progressive symptoms.

At Saharanpur again (September–November, 1930), there were twenty-eight cases, with seven recorded deaths, although the report ends with many cases showing 'no improvement'. It is not known how many of these were ultimately destroyed. Eleven horses showed cerebral symptoms, the majority of which developed from early symptoms of paraplegia. Only one was, at the outset, affected with meningo-encephalitis.

Most of the paraplegia cases commenced with severe dullness, marked lameness in one limb, either fore or hind, quickly followed by incoordination, usually posterior but occasionally anterior, with marked abduction. Several cases showed acute jaundice, while symptoms of colic in some and catarrhal fever in one, ushered in the lameness. A prefebrile stage was noted in several cases.

In 1932 (November and December), at Sargodha, twenty two fillies went down with 'paraplegia'. *Four fillies had arrived six weeks previously from Saharanpur.* The cases ranged from mild to severe. Seven animals were affected with cerebral symptoms, six of which developed rapidly from early paraplegia, and the temperature of four rose markedly ( $106.5^{\circ}$ ) before death. The remaining cases, all of which were relatively mild, were considered cured at the end of a month.

At Lahore (September, 1935—January, 1936), twenty-two cases occurred among the horses of the 6th D. C. O. Lancers. Only one animal showed acute cerebral symptoms and died. Routine temperature taking was adopted at the commencement of the outbreak. Twelve cases developed fever of four to five days duration and two of these manifested paraplegia immediately on abatement of fever.

The specific symptoms noted in this series of outbreaks were paraplegia or cerebral meningitis, some of the latter developing from the former. It may be adduced, therefore, that the disease exists in three forms, cerebral, spinal and mixed cerebro-spinal.

In mild outbreaks, the affection is spinal and the symptoms are paraplegia, i.e. paralysis or incoordination of the hind quarters, obviously dependent on involvement of the lower part of the spinal cord. It may be considered that this structure becomes progressively involved, till the higher centres are reached, with the eventual development of meningo-encephalitis. It may just as easily be argued that the brain and the cord are affected simultaneously but that the brain lesions develop more slowly, and, accepting this hypothesis, which is borne out on later pathological examinations, the cerebral lesions cannot be explained on the basis of an ascending infection from the cord.

Among the general symptoms noted may be mentioned colic, nasal catarrh, retention or incontinence of urine, weakness in the loins, loss of sensation to forceful pin-pricks in the posterior (occasionally anterior) limbs and most frequently the perineal region, pyrexia, jaundice, 'fits' and delirium.

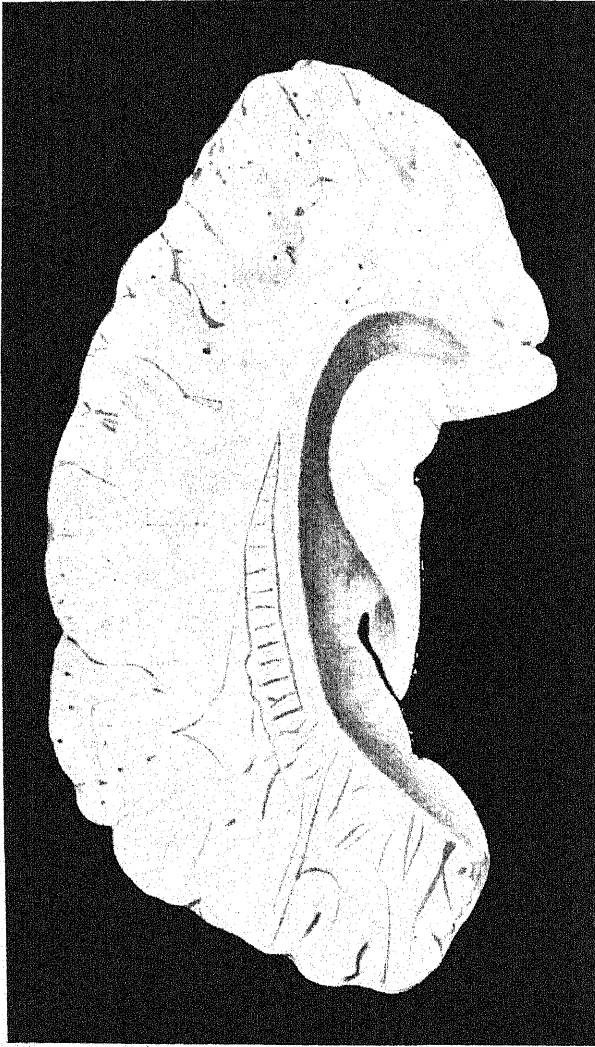
In the outbreaks recorded, all these symptoms were noted but they were most irregularly distributed among the affected horses, any of which might show only one of the above symptoms, while others might evince a combination of several. It is this peculiar feature of the symptomatology which has, apparently, baffled so many observers in the past and which certainly entitles equine encephalomyelitis to be termed a disease with a symptom complex.

It will also be observed that these outbreaks possessed a definite seasonal incidence. They all commenced at the beginning of the cold weather, when the variation between night and day temperatures is very considerable and accompanied by humidity and damp, usually with cloudy, overcast skies.

Valuable as these observations are, it was not until the Multan outbreak (1933-34) that the syndrome of equine encephalomyelitis was intensively







MESIAL SECTION OF CEREBRUM

Note the prominent haemorrhages, and the yellowish coloration of the substance. This is fairly typical of equine encephalomyelitis and is produced by dispersal of haemoglobin derivatives consequent on venous haemorrhage or suffusion of blood through over-distension of veins

studied. The exact nature of the disease was only determined during the latter part of the outbreak. Individual cases were then observed with an added knowledge of what to look for. Heane has excellently described the epizootology and symptomatology in general, the first concise account of the disease in India.

His observations may be amplified by the writer's account of the symptoms shown in fourteen cases of which he has accurate clinical and post-mortem records. An analysis of these may be of interest to show the peculiar variations of the symptom-complex.

*Horse 156.*—Found down in morning—completely paralysed and comatose. No prodromal symptoms noted, but possibly missed, as this was one of the earliest cases.

*Horse 159.*—Very sudden in onset, staggering about blindly, falling and rising again with great difficulty; watery discharge from nose; twitching of facial muscles; contraction of pupil of left eye; hyperæsthesia and tendency to fits; retention of urine and fæces. Two days illness.

*Horse 332.*—Had undergone a mild attack six weeks previously; a hopeless case; complete general paralysis and comatose. One day's illness.

*Horse 282.*—Slight weakness in hind limbs, next morning unable to rise. Loss of sensation in anterior and posterior quarters; cervical muscles rigid; fibrillar twitching of groups of muscles; conjunctiva jaundiced; pupils strongly contracted, with drooping lids (ptosis); trismus of jaws; later, Cheyne-Stokes-respiration. Destroyed as hopeless same evening.

*Horse 149.*—Very sudden onset, delirium and mania, impossible to approach, complete general paralysis supervening in a few hours. Destroyed. Petechiæ noticed dirty and jaundiced after death.

*Horse 42.*—Started off as a case of mild paraplegia. Twenty-four hours later, cerebral symptoms, unable to stand even in sling. Spasmodic contraction of cervical muscles; later, retraction of neck and rigidity; pupils, marked contractions with ptosis; hyperæsthesia and trembling of groups of muscles; grinding of teeth.

*Horse 513.*—Commenced with shivering and loss of equilibrium; sudden onset; hemiplegia; conjunctiva dirty and jaundiced; pupils normal; twitching of muscles of eyelids and lips; trismus of jaws; hyperæsthesia; great excitability; turning in circles on right side. Fell—struggling and fighting with fore-limbs; constant neighing. Destroyed.

*Horse 88.*—Admitted as a case of paraplegia. Twenty four hours later, marked hyperæsthesia, pupils contracted, collapsed in slings, Cheyne-Stokes respiration; insensitive to prick in posterior quarters, but normal anterior; no struggling; conjunctiva jaundiced. Destroyed.

*Horse 103.*—Commenced as a mild case of paraplegia and within two days, without any warning, fell down completely paralysed and quickly comatose. Destroyed.

*Horse 515.*—Slight paraplegia; twitching of muscles of face, ears, eyelids and lips; no hyperæsthesia or excitement; paralysis of lips; posterior half of body quite devoid of sensation; and anterior half, sensation dulled. Destroyed.

*Horse 266.*—Admitted as a fever case ; suspected for biliary fever ; conjunctiva jaundiced ; cedematous swelling in all limbs ; later, slight paraplegia ; found down in morning of third day, completely paralysed. Destroyed.

*Horse 416.*—Suddenly developed symptoms of acute meningitis without any warning ; maniacal frenzy. Destroyed.

*Horse 286.*—Paraplegia ; temperature  $103.2^{\circ}$  ; later, restlessness, pawing the ground ; suddenly fell and unable to rise ; cervical rigidity ; salivation ; Cheyne-Stokes respiration developed ; no sensation in posterior parts ; contracted pupils ; total paralysis. Destroyed.

*Horse 312.*—Admitted paraplegia at Khanewal, 25th November 1934, and discharged as cured on 5th February 1934. Severe recurrence with cerebral symptoms, 24th February 1934. Destroyed.

Among the general symptoms noted in these cases was a retention of urine or incontinence, the former especially in mares, the latter in males, the urine dribbling away when the animal was made to move. In males, paralysis of the retractor muscles of the penis was usual ; in mares, cedema of the vulva and some sexual excitement.

The majority of cases were ushered in by paraplegia. The greater number of these did not progress beyond this symptom and many of them were later discharged as cured. In certain of these cases, however, the ocular reflexes were abnormal and slight twitching of the facial muscles with occasional grinding of the teeth could be observed, thus indicating that the higher brain centres were involved, even if the symptoms appeared mainly spinal in origin. In all paraplegic cases, however, mild, there was an inability to maintain balance—as Heane puts it 'the animal appeared to be feeling for the ground'—a sure indication of cerebellar involvement. The writer feels convinced, on his own clinical examinations, that no case is ever purely spinal, no matter how mild the paraplegic symptoms. *The brain is always affected at the same time as the cord*, an observation which was confirmed by later pathological examinations.

Other symptoms in common, noted in this outbreak, were :—marked depression at the commencement, some degree of loss of sensation in the posterior quarters, especially over the perineum, and, perhaps one of the most important, even in mild paraplegic cases, irregularities in the heart beat, with long respiratory pauses ; fits of yawning ; in short, clinical signs of irritation of the root of the tenth cranial nerve. 'Sinking of the loins' appeared a constant symptom in all cases. Blood in the urine (hæmoglobinuria) was noticed in several cases.

The fact that stands out on a clinical examination of this, as in other, earlier outbreaks, is that there is no specific syndrome. Each case behaves somewhat differently, the possibilities of symptomatology being permutated, as it were, in each case. Only one or two symptoms may be present in the milder cases, and unless such cases occurred during the course of an outbreak, one might easily be puzzled in diagnosis of sporadic cases, or of those cases which often occur, at infrequent intervals, at the commencement of an outbreak.

(b) The difficulties of diagnosis in atypical cases are many, and a large number, if not the majority of sporadic cases, are atypical, presenting only

one or two symptoms which may be common to other diseases. Diagnosis in these cases must, eventually, unless the disease develops further, depend on pathological examination and demonstration of specific lesions in the central nervous system.

Equine encephalomyelitis, like rabies, follows no precise syndrome, even if text-book descriptions might lead one to believe so. Every veterinary officer in India knows how frequently cases of atypical rabies are encountered and how easily a mistaken diagnosis may be made. These remarks apply equally, if not more forcibly, to sporadic cases of equine encephalomyelitis. The writer, however, looks upon the examination of the urine—which *must be examined while fresh*—by the guaiacum or spectroscopic method, as a practically infallible means of diagnosing atypical, sporadic cases. Blood is always found and bile pigment only slightly less frequently.

As an instance of the difficulties in diagnosis encountered in a typical sporadic case, the following case may be cited. A horse was admitted at night—sporadic cases are usually, for some reason, manifest at nightfall—suffering from what was diagnosed as acute nephritis. In the morning, it was down and comatose, with signs around it of struggling during the night. The writer examined the kidneys and could detect no evidence of nephritis. There were, however, renal lesions which he has come to consider as specific of equine encephalomyelitis. This animal represented what was, undoubtedly, an atypical case of the disease under review. It is unfortunate that nerve tissues were not available for examination.

#### V.—PATHOLOGICAL STUDIES

Earlier observers commented on the comparative absence of lesions in this disease. Some mention was made of gastro-enteritis as a fairly constant feature and it is probably due to this that a toxicological theory of causation found favour. In a few instances, hæmorrhages in the brain or cord were noted. The complete absence of lesions was regarded by many as the peculiar feature of the disease.

The writer, in his original report (1934), briefly enumerated the lesions which he encountered in his investigations at Multan. A critical analysis of the protocols of the fourteen cases referred to gives an indication of the variability of lesions which may be encountered, even in peracute clinical cases of the same type.

*Horse 156.*—Congestion of meningeal vessels of brain and cord. Punctate hæmorrhages in grey matter. Kidneys congested. Patchy enteritis, small intestine.

*Horse 159.*—Mild enteritis; renal congestion; spleen slightly enlarged; congestion of meningeal vessels; punctate hæmorrhages in grey matter of brain and cord. *Streptococcus melanogenes* isolated.

*Horse 332.*—Blood shows little tendency to coagulate; intense congestion of meningeal vessels over cerebral cortex, cerebellum and cord; spleen considerably enlarged; mucous membrane of urinary bladder highly congested; slight cystitis; kidneys congested; mild congestion of gastro-intestinal tract.



*Horse 282.*—Similar to foregoing. Blood clots normally, but darker in colour. Urinary bladder hæmorrhagic. Acute cystitis.

*Horse 149.*—Gastro-enteritis, mild. Vessels of cerebrum and cerebellum, highly congested; spinal meningeal vessels—slightly congested in a patchy manner. Liver, kidneys and spleen congested, the spleen pulp softer than normal; slight petechiæ on endocardium and on bladder mucosa. Slight cystitis. *Streptococcus melanogenes* isolated.

*Horse 42.*—Slight gastro-enteritis; congestion of meningeal vessels extensive, brain and cord; severe hæmorrhage into pial folds of cerebral cortex, both sides, and cerebellum and at base of brain; hæmorrhages throughout the surface, and brain pulp has a peculiar yellowish colour and somewhat softened; gelatinous œdema, cervical and lumbar parts of cord. Kidneys congested; bladder, much thickening and hæmorrhage; chronic cystitis. Spleen normal. Liver, congested.

*Horse 513.*—Catarrhal inflammation of small intestines; congestion of vessels, cerebral cortex and cerebellum; extensive hæmorrhage near the thalamus; brain pulp decidedly yellow in colour but not softened; meningeal vessels of cord congested, with hæmorrhage in lumbar region, and jelly-like material between the pia and dura mater. Urinary bladder, hæmorrhagic, full of blood. Mild cystitis. Spleen—"like an anthrax spleen". Lymph glands, dark and hæmorrhagic. Liver, markedly icteric. Blood tarry and fails to clot. Kidneys, severely hæmorrhagic.

*Horse 88.*—General gastro-enteritis; congestion of meningeal vessels, cerebrum and cerebellum; spinal, meninges intensely congested with patchy hæmorrhage of considerable depth. Spleen slightly congested. Urinary bladder normal. Kidneys and liver congested.

*Horse 103.*—No inflammation gastro-intestinal tract; severe congestion meningeal vessels of cerebrum and cerebellum; hæmorrhages in pulp prominent with general reddening; spinal cord, slight congestion of meningeal vessels; severe congestion of spleen and liver with hæmorrhages in the substance; kidneys full of blood; urinary bladder, few petechiæ.

*Horse 515.*—Mild gastro-enteritis; congestion of meningeal vessels of cerebrum and cerebellum slight; gelatinous exudate around the cord throughout; urinary bladder, mild cystitis; kidneys congested.

*Horse 266.*—No enteritis; meningeal vessels of cerebrum and cerebellum highly congested; gelatinous exudate over cerebral cortex—meningitis; spinal cord, meningeal vessels slightly congested; spleen, enlarged and markedly congested; kidneys, congested; urinary bladder, a few petechiæ. Cystitis *Streptococcus melanogenes* isolated.

*Horse 416.*—Mild gastritis; intestines normal; blood clots poorly; congestion of meningeal vessels, cerebrum and cerebellum; vessels of spinal meninges, highly congested; jelly-like material extensive around the cord; lymph glands swollen and hæmorrhagic; spleen, slightly enlarged and hæmorrhagic, kidneys congested; urinary bladder, few petechiæ. *Streptococcus melanogenes* isolated.

*Horse 286.*—Slight muco-enteritis; severe congestion meningeal vessels cerebrum and cerebellum, with definite meningeal œdema—meningitis; hæmorrhage around cerebellar peduncles; spinal cord, gelatinous œdema

extensive. Liver and kidneys highly congested; bladder, a few petechiæ; spleen, few surface petechiæ and hæmorrhagic. Kidney, highly congested; lymph glands hæmorrhagic; blood clots poorly.

*Horse 312.*—No enteritis; definite meningitis, slight adhesions between pia mater over entire cerebral cortex and cerebellum; pia firmly adherent to cerebral substance; slightly gelatinous, somewhat organized, exudate; cord, congestion of meningeal vessels; kidneys markedly congested; spleen, slightly enlarged and darker in colour; liver mildly congested; urinary bladder normal.

These cases were all peracute. It will be seen that there was a diversity of lesions both in the internal organs and central nervous system, although they all conformed to a general type. Apart from the constant, yet variable, lesions of the brain and cord, the lesions of the internal organs, with the exception of the kidney, were not uniformly present. Neither gastro-enteritis, acute splenitis, failure of the blood to clot, acute hæmorrhagic lymphadenitis, hæmorrhagic cystitis, nor hæmorrhage in the parenchymatous tissues could be looked upon as a constant feature. It is difficult, if not impossible, to account for the presence of these lesions in some cases and not in others.

*Streptococcus melanogenes* (Schlegel) was isolated from the cerebral tissue of two cases, from the cerebral cortex and blood of one case and from the blood alone in another case. When the fact was observed, in the earliest case, it was considered that this organism might be significant, as a secondary invader, in the development of 'septicæmic' lesions. This hypothesis was quickly dispelled when it was found impossible to isolate it with constancy and when it appeared in cases which were definitely non-"septicæmic" in type. No pathological significance, even of a secondary nature could, therefore, be attributed to this organism.

No matter what the intensity of the cerebral and spinal lesions, hæmorrhage, either punctiform or extending to a depth of several millimeters, was always present in the grey matter, throughout the cerebral cortex, the cerebellum, the brain stem and the cord. This was undoubtedly a constant feature. The cut surface of the brain was, usually, yellowish in colour or markedly reddened.

The liver of all cases showed some degree, however mild, of icteric staining and parenchymatous degeneration. The liver is not so commonly affected with vascular changes, although occasionally hæmorrhages in the substance are found, and the myocardium and the lung substance may be similarly affected.

In several acute and subacute cases whose post-mortem examination the writer witnessed, the writer came to the conclusion that the constant lesions are:—congestion of the meningeal vessels, with loss of lustre of the meninges and some mild thickening, both of the brain and cord; icteric discolouration of the liver; hæmorrhages of varying degree, usually punctate, of the grey matter, and renal congestion. The meningeal vessels appear peculiarly sclerotic in all cases.

Cystitis appears, in a certain number of cases. There is obviously present, in every case, an early paralysis of the bladder (catheterization is always necessary). Some horses, as the result of retention, rapidly develop cystitis.

Others do not, and cystitis cannot, therefore, be looked upon as a primary lesion of the disease but as essentially secondary. The degree of cystitis which is present in some cases, the marked induration of the bladder wall and the early formation of calculus, force one to the conclusion that the vesical paralysis and retention must have been insidious in its development and even in peracute cases, apparently normal a few hours previously, which suddenly became paralysed and died within twenty-four hours, chronic cystitis may be found. A reasonable explanation of this is that these cases had been undergoing a slow, insidious, progressive invasion of and damage to vital nerve centres and it was only when the infection reached the brain in a massive concentration, or when the damage wrought on the cortical tissues reached a peak, that a crisis was precipitated with the sudden appearance of peracute symptoms. This is not an unreasonable assumption when one considers that, in the subacute and chronic cases the cord is predominantly affected and the cerebral lesions extremely slight, though definite.

In spite of the marked cystitis frequently present, no case of ascending pyelitis has ever been encountered. Marked swelling of the spleen appears a fairly constant lesion. Hæmorrhage from the splenic vessels is occasionally severe and coalescence of such areas results in the transformation of the spleen into a vast blood clot with obliteration of the cellular elements.

The kidney lesion is of particular interest. The kidney is enlarged, the capsule tense, but the substance is not pale in colour or somewhat mottled as in an acute parenchymatous nephritis. The cut surface possesses a uniform colour and lines of hæmorrhage may be seen radiating between the tubules, both of the medulla and cortex. The arterial branches in the pyramids are sclerotic. This is such a constant lesion in the chronic form of the disease as to be practically diagnostic when the lesions affecting the central nervous system are slight, and there is a strong clinical history.

In subacute cases the lesions may be so slight as to escape notice. The only lesions may be a ground glass appearance of the meningeal vessels, and punctate hæmorrhages in the grey matter of the brain and spinal cord. These, as well as the faint hæmorrhagic intertubular striæ in the kidney, may only be visible with a hand lens. Out of nineteen samples of urine examined, all were positive for blood and fifteen for bile pigment. The cerebro-spinal fluid was invariably clear and appeared under pressure.

The difficulties in evaluation of pathological findings during the course of an outbreak is well illustrated in the Veterinary Officer's experiences during the outbreak at Sargodha, 1932. Of the seven fillies which died, filly 7804 showed a complete loss of sensation in the quarters and in the perineum and certain brain symptoms were detected. At post-mortem examination, the blood was tarry and fluid, the blood vessels of the brain and cord showed intense congestion and numerous petechiæ were present on the bladder mucosa and the intestine. The temperature of this filly rose to 104° before death. Filly 8054, ill for five days, showed no other symptom than incoordination and the only lesion noticed at post-mortem was slight enlargement of the spleen. Filly 8686, ill for the same period, was similarly affected with slight intestinal congestion in addition. In both these cases the temperature rose before death. Filly 8992 showed no lesions at post-mortem. Filly 7987 showed





distribution of these lesions is irregular throughout the brain, relatively slightly affected areas existing alongside of areas in which the changes are expressed in maximum degree, even in adjacent areas of the parietal cortex. In the cord, there is no particular localisation of this process. The dorsal and ventral horns are equally affected, and the lesion is, therefore, unlike that of anterior poliomyelitis in which the anterior horn is alone affected.

In no case has it been possible to demonstrate the presence of specific inclusion bodies, whether intranuclear or cytoplasmic, in the nerve cells of Ammon's horn, which is a selective site of occurrence [Nicolau and Galloway.] Bouin was used as a routine fixative, on account of its rapid penetration. According to certain observers [Hurst], sublimate fixed material is to be preferred in examining for inclusion bodies and this observation coincides with the writer's later experiences in the examination of nerve tissues for Negri bodies. The routine stain used was Mann, but the panoptic method, May-Grunwald-Giemsa and Pappenheim, gave equally negative results. It appears, therefore, at the moment, that 'inclusion bodies' are not a specific feature of the Indian form of equine encephalomyelitis and it is this feature which promptly distinguishes it from 'Borna disease'.

The blood vessels, especially the arterioles, of the brain and cord are affected with a chronic, proliferative type of inflammation extending through all coats and leading to a narrowing of the lumen. The gross thickening of the blood vessels is a characteristic lesion. One would expect, in such lesions, to find thrombosis fairly frequent, but this lesion has never been noted and areas of infarction with secondary softening (colliquative necrosis) are certainly absent. The veins are abnormally distended and over dilatation tends to spontaneous rupture with considerable haemorrhage. It is not unusual to find, especially in the vessels of the pia-arachnoid, an extensive area of venous haemorrhage surrounding the homonymous artery and, in the cord, effecting a mechanical separation of the dorsal nerve ganglia.

The kidney lesion consists, in early cases, of marked haemorrhage between the tubules, which themselves are perfectly normal, although they contain a considerable quantity of blood. Rupture of the capillaries of the glomerular tuft is frequent. This lesion appears to depend on a primary sclerosis of all branches of the arterial supply of the kidney. It is interesting to note that Nicolau and Galloway encountered a similar lesion in the kidneys of inoculated rabbits. "The condition may be described as renal congestion, not a true nephritis", and they consider it to originate from a compression of blood vessels. In cases which have survived for a long time, there is a marked tendency for an interstitial change to supervene although haemorrhages are still prominent. Perivascular infiltration is frequently seen around the small cortical vessels. Similar changes affecting the blood vessels may be seen in the spleen and liver and, less frequently, in the lungs, and haemorrhage in these organs has undoubtedly a similar origin to that noted in the kidney.

#### VII.—THE SEARCH FOR A NEUROTROPIC VIRUS

Mention has been made, in a preliminary note, of efforts to determine the etiological factor in this disease. It has long been an impression in India

that equine encephalomyelitis is caused by the ingestion of toxic forage and this idea of fodder poisoning is one that is still held in many quarters. This theory may be briefly disposed of by stating that:—

- (a) Examination of the stomach ingesta of all horses succumbing to the disease was negative for group alkaloid test.
- (b) A series of rabbits and guinea-pigs fed on these samples *ad lib.* remained free from any symptom, toxic or otherwise.
- (c) The withholding of suspected forage and its replacement by fresh supplies did not result in any cessation of the disease.
- (d) Samples of forage appeared perfectly sound and were free from mouldy decomposition and poisonous seeds, (e.g. *Abrus precatorius*). The feed was carefully balanced and there was no probability of a cereal (phytic acid) poisoning.
- (e) The grasses and grains fed to horses during the Multan outbreak were examined by competent agricultural botanists who could detect no potentially poisonous species.
- (f) The same forage from exactly the same source of supply, was fed to other cavalry regiments, which remained entirely free from disease.
- (g) There is no proof that forage poisoning, whatever that term may mean, can be responsible for pathological lesions in the central nervous system of the type described.

The toxic etiology of the disease was one seriously considered by the writer and finally abandoned in favour of a virus theory, in view of the fact that the disease on epizootological and pathological grounds was strictly comparable with a variety of forms of equine encephalomyelitis, in many of which the existence of a virus has been proved.

The earliest experiments were performed on horses, aged army casters being the only animals available, and in rabbits and guinea-pigs. The results of a successful subdural transmission in horses has been recorded [Mosley, Hean and Shirlaw, 1934]. The clinical syndrome of these two cases was typical of the affection. On post-mortem examination, specific lesions of equine encephalomyelitis were found in the central nervous system and the kidney. Mention has also been made of the failure to reproduce the disease in horses by intrathecal inoculation of brain emulsion. This failure was anticipated. The resistance to passage of virus particles on part of the meningo-encephalic barrier is well known.

The first series of rabbits and guinea-pigs was inoculated as follows:—

Small portions of brain were removed immediately after destruction of hopeless cases (Multan outbreak) and rushed to the laboratory at Lahore in thermos flasks packed with ice. The tissue invariably arrived in a frozen state and small pieces of representative areas of the brain were quickly triturated in saline and sterile sand. After being allowed to settle in the refrigerator for eight hours, the supernatant fluid was aspirated. This constituted the inoculum. Rabbits and guinea-pigs, two for each specimen, were trephined and subdurally inoculated with 0.1 c.c. of the inoculum, the procedure being the same as for the propagation of rabies virus in the manufacture of Semple's vaccine.

The mortality due to the technique was nil. Several guinea-pigs and rabbits died within a period of three to eight days, but as they showed no nervous symptoms, it was not considered, at that time, that a specific encephalomyelitis had been transmitted. The brain and cord were, fortunately, removed and fixed in each case. It was a surprise, therefore, to find, on histopathological examination of the brain and spinal cord of these cases, precise lesions of encephalomyelitis and this finding naturally led to the planning of more carefully observed experiments.

It proved easy to obtain fresh inoculation material, as from this time, reports of sporadic cases of equine encephalomyelitis were frequent.

### EXPERIMENTAL

#### *Transmission Experiments : material—Multan outbreak*

(All inoculations consisted of 0.1 c.c. hippocampus major emulsion—unless otherwise stated)

	Source of inoculum	Date of inoculation	Period observed	Result
Guinea pig 110	5 c.c. spinal fluid from Horse 627, subcut.	26-11-33	26-1-34	No reaction
Rabbit 109	Do.	Do.	Do.	Do.
Rabbit 150	Horse 288	25-1-34	29-1-34	Died—E. E.
Rabbit 151	Horse 288	25-1-34	29-1-34	Do.
Rabbit 154	Rabbit 151	29-1-34	1-2-34	Died.
Guinea pig 159	Horse 515	30-1-34	2-2-34	Died—E. E.
Rabbit 155	Rabbit 151	29-1-34	2-2-34	Do.
Rabbit 156	Horse 515	30-1-34	3-2-34	Do.
Rabbit 173	Horse 286	11-2-34	13-2-34	Do.
Rabbit 170	Horse 416	9-2-34	17-2-34	Do.
Guinea pig 158	Horse 515	30-1-34	1-3-34	Do.
Rabbit 172	Horse 286	11-2-34	27-3-34	No reaction
Rabbit 157	Horse 515	30-1-34	27-3-34	Do.
Rabbit 140	Horse 84	11-1-34	27-3-34	Do.
Rabbit 141	Horse 84	11-1-34	27-3-34	Do.
Rabbit 148	Horse 154	22-1-34	27-3-34	Do.
Rabbit 169	Horse 416	9-2-34	27-3-34	Do.
Rabbit 149	Horse 154	22-1-34	27-3-34	Do.

These results appear somewhat inconclusive. Fifteen rabbits were inoculated and only seven reacted. None showed any nervous symptoms, yet all were completely flaccid and recumbent several hours before death, with absence of all reflexes. The criticism may be made that too short an interval elapsed between the inoculation and death. The average period was four and a half days. The fact remains, however, that none of these rabbits showed, on post-mortem examination, any sign of injury to the brain as the result of inoculation. The brain and spinal cord of these animals were severally affected with lesions of encephalomyelitis identical with those seen in the subjects whence the inoculum was derived, and haemorrhagic lesions, with no involvement of the parenchyma, were noticed in the kidney. The fact that only seven rabbits reacted, in face of what must be considered



a virulent inoculum, cannot be explained. It may be mentioned that no case of spontaneous occurrence of a specific neurotropic virus infection had ever been noted in the rabbit stock. No group virus immunity could explain the phenomenon. The supposition that a mild degree of cerebral irritation had been induced with a resultant flaring up of a latent but specific virus is one that had no support.

The results of guinea-pig inoculations correspond in all essential details with those in rabbits. Two out of three guinea-pigs died with similar and more intense lesions than those noted in rabbits. It will be seen that both guinea-pigs injected subdurally died, with an average period between inoculation and death of three days. The lesions in the spinal cords of both guinea-pigs were peracute and the inflammation was entirely monocellular. It will also be noted that one serial transmission was made, with successful result between rabbits 151 and 155.

The second set of transmission experiments was carried out with materials from three horses which developed paraplegia some months after the cessation of the main outbreak affecting the horses of the 13th D. C. O. Lancers, and after the regiment, stationed at Jullundur, had been declared free of the disease.

Horse 62 developed paraplegia at Khanewal on 7th December 1933 and made an apparent recovery. On 4th October 1934 this animal showed complete incoordination of the posterior limbs with incontinence of urine, which progressed till destruction was necessitated.

Horse 233 was a similar case. Pyrexia was noted at Multan on 30th January 1934 and this animal was discharged cured three days later. On 4th October 1934 (the date of a general veterinary inspection of the regiment) lack of coordination and incontinence of urine was noted.

Horse 527 developed paraplegia at Khanewal on 24th November 1933 and was a precisely comparable case. These three animals were destroyed at Lahore and the brain and cord used for inoculation experiments in rabbits and guinea-pigs. The technique employed was that of Nicolau and Galloway in their studies of Bornâ disease.

*Transmission Experiments: material—Jullundur cases*

(All inoculations were made subdurally with 0.1 c.c. hippocampus major emulsion)

	Source of inoculum	Date of inoculation	Period observed	Result
Rabbit 197 . . . . .	Horse 62 . . . . .	21-1-35	22-3-35	Lived.
Guinea pig 198 . . . . .	Do. . . . .	21-1-35	24-1-35	Died—traumatic meningitis.
Rabbit 199 . . . . .	Do. . . . .	21-1-35	24-1-35	Died—Pneumonia and enteritis. E. E. negative.
Guinea pig 201 . . . . .	Do. . . . .	21-1-35	16-2-35 (25 days).	Died—E. E.
Rabbit 200 . . . . .	Do. . . . .	21-1-35	22-3-35	Lived.



*Transmission Experiments: material—Jullundur cases—contd*

---	Source of inoculum	Date of inoculation	Period observed	Result
Guinea pig 202 . . . . .	Horse 62 . . . . .	21-1-35	25-2-35 (33 days).	Died—E. E.
Rabbit 229 . . . . .	Horse 233 . . . . .	19-2-35	1-5-35	Lived.
Rabbit 230 . . . . .	Do . . . . .	19-2-35	1-5-35	Do.
Guinea pig 227 . . . . .	Do. . . . .	19-2-35	13-3-35 (22 days).	Died—E. E.
Guinea pig 228 . . . . .	Do. . . . .	19-2-35	14-3-35 (23 days).	Do.
Rabbit 341 . . . . .	G. P. 227 . . . . .	13-3-35	25-5-35	Lived.
Rabbit 342 . . . . .	Do. . . . .	13-3-35	25-5-35	Do.
Rabbit 327 . . . . .	Horse 527 . . . . .	7-3-35	1-6-35	Do.
Rabbit 328 . . . . .	Do. . . . .	7-3-35	1-6-35	Do.
Guinea pig 329 . . . . .	Do. . . . .	7-3-35	5-4-35 (30 days).	Died—E. E.
Guinea pig 330 . . . . .	Do. . . . .	7-3-35	5-4-35 (30 days).	Do.
Rabbit 331 . . . . .	Horse 527 (with filtered Berkfield V emulsion).	7-3-35	1-6-35	Lived.
Rabbit 332 . . . . .	Do. . . . .	7-3-35	1-6-35	Do.
Guinea pig 333 . . . . .	Do. . . . .	7-3-35	3-4-35 (28 days).	Died—E. E.
Guinea pig 334 . . . . .	Do. . . . .	7-3-35	7-4-35 (32 days).	Do.

The brain tissue, hippocampus major, of Horse 527 was refrigerated for one week at  $-5^{\circ}\text{C}$ . and the following transmissions were effected with 0.1 c.c. of the emulsion, subdurally.)

	Date of inoculation	Period observed	Result
Rabbit 337 . . . . .	15-3-35 (unfiltered).	1-6-35	Lived.
Rabbit 338 . . . . .	15-3-35 (filtered).	1-6-35	Do.
Guinea pig 339 . . . . .	15-3-35 (unfiltered).	7-4-35 (23 days).	Died—E. E.
Guinea pig 340 . . . . .	15-3-35 (filtered).	9-4-35 (25 days).	Do.

The concluding set of experiments of this nature were conducted with brain and cord material received from three horses (A-40, A-84 and C-318) which died during an outbreak of typical equine encephalomyelitis (mild) which occurred in the horses of the 7th Light Cavalry stationed at Loralai during the winter of 1935-36.

